STUDY OF BACTERIAL AND PARASITIC PROFILE OF ACUTE DIARRHOEA IN CHILDREN UNDER FIVE YEARS OF AGE WITH SPECIAL REFERENCE TO DETECTION AND CHARACTERIZATION OF ROTA VIRUS

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CERTIFICATE

This is to certify that this dissertation entitled "**STUDY OF BACTERIAL AND PARASITIC PROFILE OF ACUTE DIARRHOEA IN CHILDREN UNDER FIVE YEARS OF AGE WITH SPECIAL REFERENCE TO DETECTION AND CHARACTERIZATION OF ROTA VIRUS"** is the bonafide work done by **Dr.K.SUBHA** in the Department of Microbiology, Govt. Stanley Medical College & Hospital, Chennai, in partial fulfillment of the regulation for **M.D. (Branch - IV) Microbiology** examination of the Tamil Nadu Dr.M.G.R.Medical University, Chennai, to be held in April 2012.

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DECLARATION

I, **Dr.K.SUBHA,** solemnly declare that this dissertation **"STUDY OF BACTERIAL AND PARASITIC PROFILE OF ACUTE DIARRHOEA IN CHILDREN UNDER FIVE YEARS OF AGE WITH SPECIAL REFERENCE TO DETECTION AND CHARACTERIZATION OF ROTA VIRUS"** is the bonafide work done by me at the Department of Microbiology, Government Stanley Medical College and Hospital, Chennai, under the guidance and supervision of **Prof.Dr.R.SELVI, M.D.,** Professor of Microbiology, Government Stanley Medical College, Chennai-600 001.

This dissertation is submitted to The Tamil Nadu Dr. M. G. R. Medical University, Chennai in partial fulfillment of the University regulations for the award of degree of M.D. Branch IV Microbiology examinations to be held in April 2012.

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INSTITUTIONAL ETHICAL COMMITTEE,
STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : Study of prevalence of Bacterial, Parasitic and rota viral Infections in children less than five years of age with Diarrhea

Principal Investigator : Dr.K.Subha

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The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 01.02.2011 at the Modernized Seminar Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

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08/11/2011

MEMBER SECRETARY,
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CONTENTS

Introduction

INTRODUCTION

The gastrointestinal tract is home to 10 times more bacteria than cells in the entire human body. It is the site of sophisticated interactions between microbial pathogens, commensals, host epithelial cells and the immune system, a kind of jungle of biologic interactions, evolution and natural history whose balance is crucial for childhood growth and development.[59]

Diarrhoea is one of the most common causes of morbidity and mortality in children worldwide. Globally, there are about two billion cases of diarrhoeal disease every year[106].Diarrhoeal disease is the second leading cause of death in children under five years of age with an estimated 1.5 million child deaths every year. It kills more young children than AIDS, malaria and measles combined[99].

Diarrhoeal disease continues to be a public health problem especially in developing countries where they are estimated to be responsible for 2.5 million infant deaths per year with an annual mortality rate of 4.9/ per 1000 children and an incidence of of 3.2 episodes of diarrhoea per child-year among children under 5 years of age. [50]

In India ,one third of total paediatric admissions in hospitals are due to diarrhoeal diseases and 17% of all deaths in indoor paediatric patients are diarrhoea related[76]. Diarrhoea is the cause of death in almost 23% of Indian children who die before the age of 5.[40]

In developing countries , diarrhoeal disease is most commonly due to infectious cause.[28] Although many number of viral , bacterial and parasitic causes of diarrhoea have been identified todate , only a few of the etiologic agents cause the vast majority of diarrhoeal disease in children in the developing world and include rotavirus, diarrhoeagenic Escherichia coli, Campylobacter jejunii, Shigella sp, nontyphoidal Salmonella ,Giardia lamblia, Cryptosporidium sp and Entamoeba histolytica.[91] Many of these organisms are transmitted easily through food or water or from one person to another and some are devastating to individuals with compromised immune system.[78]

Diarrhoeagenic Escherichia coli pathotypes represent a leading bacterial cause of paediatric diarrhoea in developing countries. The diarrhoeagenic Escherichia coli increasingly being reported in recent years is the Enterohaemorrhagic E.coli[38] .The enterohaemorrhagic E.coli cause severe disease in humans such as haemolytic uremic syndrome by the production of shiga toxin[16].

Worldwide the incidence of shigellosis is highest among children 1 to 4 years old[49]. In developing countries, shigella infections most commonly caused by S.flexneri are mainly endemics causing approximately 10% of all diarrhoeal episodes among children less than 5 years of age. It is estimated that shigella causes 164.7 million cases of diarrhoea of which 163.2 million occur in developing countries, and 1.1 million deaths each year worldwide mostly in developing countries[6]. Regarding parasitic causes, Entamoeba histolytica, Cryptosporidium, and Giardia are common in developing countries.

Rotavirus disease is the single most important cause of severe gastroenteritis in children throughout the world [3,7,67]. Globally about 30%–40% of hospitalizations and deaths due to diarrhoea among children under 5 years old, and about 5% of all child deaths, are attributed to rotavirus infection[96,26]. It occurs as a sporadic seasonal form, even as severe gastroenteritis of infants and younger children, mostly in the first 2–3 years of life, with a peak at the age of 6–24 months.[26]

Every year, rotavirus gastroenteritis is estimated to cause approximately 527,000(475,000-580,000) deaths globally among children <5 years old[107]. Of about 500,000 annual deaths due to rotavirus worldwide, more than 1,50,000 occur in India[103] and risk of rotavirus disease–associated death by 5 years of age is 1/250 in India[75].

The burden of this frequent, potentially serious childhood disease in India could be reduced by routine rotavirus vaccination of infants because the efforts to decrease the number of deaths due to diarrhoea by improving water quality and sanitation, promoting breast-feeding, and introducing treatment programs based on oral rehydration therapy have decreased the mortality rate associated with infection due to bacterial and parasitic agents but they have been less effective in reducing rotavirus disease–associated morbidity and mortality[102].

For rota viral infections, epidemiological studies can be used to select areas for vaccine trials depending upon disease burden and to serve as baselines for identification of new strains should they emerge[72].The epidemiology of rota virus in India remains in constant flux. So continuous monitoring of rota virus genotype

distribution would be valuable to show up the diversity and changes in the circulating strains.

Since optimal therapy depends on the identity of the infective agent, definitive microbiologic studies are essential[42]. The treatment of bacterial enteric infection depends on the infective agent. For example some organisms such as enterohaemorrhagic E.coli, nontyphoidal Salmonella , antimicrobial therapy is not required as it increase the complication. In contrast, enteric fever and shigellosis benefit from antimicrobial treatment.

Because laboratory tests for faecal specimens are time consuming and expensive, their inclusion in routine patient management usually is limited in nonhospitalized patients to those with severe diarrhoea. Young children are at particular risk of severe diarrhoeal disease and can benefit from appropriate laboratory support[19]. Further, identifying the enteric pathogens that contribute significantly to the causes of diarrhoea in children will help to provide an accurate and region specific estimate of disease burden[91].

Hence this study was undertaken to identify the bacterial, parasitic profile and rota viral infections in children under 5 years of age with diarrhoea and from children under 5 years of age presenting to hospital for reasons other than diarrhoeal illness.

Aims and Objective Of The Study

AIMS AND OBJECTIVES OF THE STUDY

- To determine the prevalence of bacteria and parasites in stool samples from diarrheal cases of children under 5 years of age.
- To find antibiotic susceptibility of significant bacterial isolates.
- To determine the incidence of rota virus infection in children under 5 years of age with acute diarrhoea.
- To examine the age distribution of rota virus infected children.
- To evaluate clinical severity of rota virus infection in children.
- To evaluate the protective effect of breast feeding on rota virus diarrhoea.
- To evaluate the distribution of different genotypes of rotaviruses circulating in the community.

Review Of Literature

REVIEW OF LITERATURE

The term ‘diarrhoea’ is derived from Greek and it means ‘to flow through’. It is defined as a condition characterized by an increase in the frequency, fluidity and weight of the stool, compared to the normal bowel habit of the child. Diarrhoea is usually a symptom of an infection in the intestinal tract, which was described by Hippocrates as ‘the abnormal frequency and liquidity of faecal discharge’ 2000 years back[84].

Diarrhoeal diseases are 1.one of the most common illness in infants and young children throughout the world. 2.one of the six leading causes of 10.6 million deaths that occur annually in children younger than 5 years of age 3.Account for 18% of the 10.6 million deaths with the greatest toll being in the developing countries[13].

Normal defences of the bowel include 1.Gastric acid 2.Bile salts 3.Lymphoid tissue 4.Enterokines 5.Normal bowel flora 6.Secretory immunoglobulin A 7.Motility 8.Hepatic deactivation of toxins.

Diarrhoea may be classified as ‘acute’ if it lasts for less than 2weeks, ‘persistent’ if the duration of diarrhoea is 2-4 weeks and ‘chronic’ if more than 4 weeks duration[86].

Acute diarrhoea may be due to following reasons[35]:

1. Infectious 90 %
2. Drug induced
3. Ingestion of toxins 10 %
4. Ischemia
5. Other conditions

Infectious diarrhoea may be caused by viruses, bacteria and protozoa. These agents of diarrhoea are acquired by ingestion of contaminated food or water and transmitted by faeco-oral route[97]. Infectious diarrhoea can be classified into 2 distinct clinical syndromes : inflammatory or bloody diarrhoea and noninflammatory or nonbloody diarrhoea[22]. Inflammatory diarrhoeas produce severe form of acute diarrhoea. In contrast, noninflammatory diarrhoeas are milder but produces severe fluid loss with attendant morbidity and mortality[51].

PATHOGENESIS[43]:

Enteric pathogens adhere to mucosal cells via fimbrial or afimbrial microbial adhesions and induce formation of cuplike pedestals on which bacteria rest. After this interaction, the pathogenesis of the bacteria depends on whether the organism remains adherent to the cell surface and elaborates a secretory toxin, invades the mucosa or penetrates the mucosa.

Pathogenesis :

Characteristic	noninflammatory [secretory,noninvasive,toxin producing]	inflammatory [mucosal,invasive]	penetrating [systemic]
Mechanism	Activation of adenyl cyclase[34], Activation of guanylate cyclase, Alteration of intracellular calcium, Stimulation of enteric nervous system, Loss of brush border hydrolase activity, Unopposed crypt cell secretion[20]	Invasion, Cytotoxin	Penetrating
Location[57]	Proximal small bowel	Colon	Distalsmallbowel
Illness	Watery diarrhoea	Dysentery	Enteric fever
Stool examination	No faecal leucocytes Mild or no ↑lactoferrin	Faecalpolymorpho nuclear leukocytes ↑↑lactoferrin	Faecal mononuclear leukocytes
Etiological agents	Vibrio cholerae, Escherichia coli(ETEC*) Clostridium Perfringens Bacillus cereus Staphlococcus aureus Giardia lamblia Rota virus Norwalk like viruses Cryptosporidium parvum E.coli(EPEC,EAggEC)* Microsporidia Cyclospora cayetanensis	Shigella E.coli(EIEC,EHEC)* Salmonella enteriditis Vibrio parahemolyticus Clostridium difficile Campylobacter jejuni Entamoeba histolytica	Salmonella typhi Yersinia enterocolitica

* ETECEnterotoxigenicE.coli, EHECEnterohaemorrhagicE.coli,

EIEC-EnteroinvasiveE.coli, EPEC-EnteropathogenicE.col,

EAggEC-Enteroaggregative E.coli

The causes of acute diarrhoea in children are[27]:

Viruses:(70%)	Bacteria:(10-20%)	Parasites
Rota viruses Noroviruses(Norwalk like viruses) Enteric adeno viruses Calciviruses Astro viruses	EnteropathogenicEscherichia coli Shigella spp Nontyphoid Salmonella spp Campylobacter jejuni Yersinia enterocolitica Shigatoxin producing E.coli Salmonella typhi and Salmonella paratyphi Vibrio cholerae	Protozoa: Cryptosporidium Giardia lamblia Entamoeba histolytica Helminths: Strongyloides stercoralis

VIRAL GASTRO ENTERITIS:

Viral gastro enteritis is a global problem in infants and young children[9]. Viruses were long suspected to cause gastro enteritis but efforts to demonstrate specific agents were not successful until the 1970s when the Norwalk agent was first observed by electron microscopy in negatively stained stool suspensions[44].

Viruses are suspected when vomiting is prominent, the incubation period is longer than 14 hours and the entire illness is over in less than 72 hours. Viral pathogens are likely when there are no warning signs of bacterial infection and there are no epidemiologic clues from the history[22].

Viruses causing gastroenteritis[10]:

Rota viruses	-	genus Reoviridae
Noro viruses and Sapo viruses	-	genus Calciviridae
Astro viruses	-	genus Astroviridae
Enteric adeno viruses	-	group F of Adenoviridae

Incidence in Children[11,73]:

Rota viruses	30-60%
Noro viruses and Sapo viruses	8-30%
Astro viruses	6-9%
Enteric adeno viruses	3-6%

Other viruses found in the gastro intestinal tract that are not regularly associatedwithdiarrhoea:Enteroviruses, Reoviruses,Toroviruses,Coronaviruses, Parvoviruses. Human immune deficiency virus can infect the gut directly.

Viruses which infect the gut under conditions of immune suppression: Herpes simplex viruses, Cytomegalovirus, Picobirnavirus.

ROTA VIRUS:

Introduction:

Rota viruses are the single most important cause of severe diarrhoeal illness in infants and young children in both developing and developed countries worldwide accounting for 30-50% of these illnesses[74].

History[27]:

In 1973, Ruth Bishop et al identified round viral particles termed 'rotavirus' by electron microscopy in duodenal biopsies of infants with gastroenteritis in Melbourne. Shortly afterward rotavirus was identified in faeces by electron microscopy by Flewett et al and Bishop et al . Although the human rotaviruses were discovered in 1973, Adams and Kraft described virus like particles in intestinal tissue of mice infected with epizootic diarrhoea of infant mice virus in 1963. These particles were similar to those observed in children by Bishop et al. In 1963, Malherbe et al described the isolation of simian agent 11 virus. In 1967, Malherbe et al described offal agent from intestinal washings of cattle and sheep. In 1969, Mebus et al reported Nebraska calf diarrhoea virus in fetal bovine cell cultures. The murine, simian, offal agent and bovine agents were later found to exhibit characteristic rota virus morphology and to share a group antigen with other rota viruses.

Epidemiology:

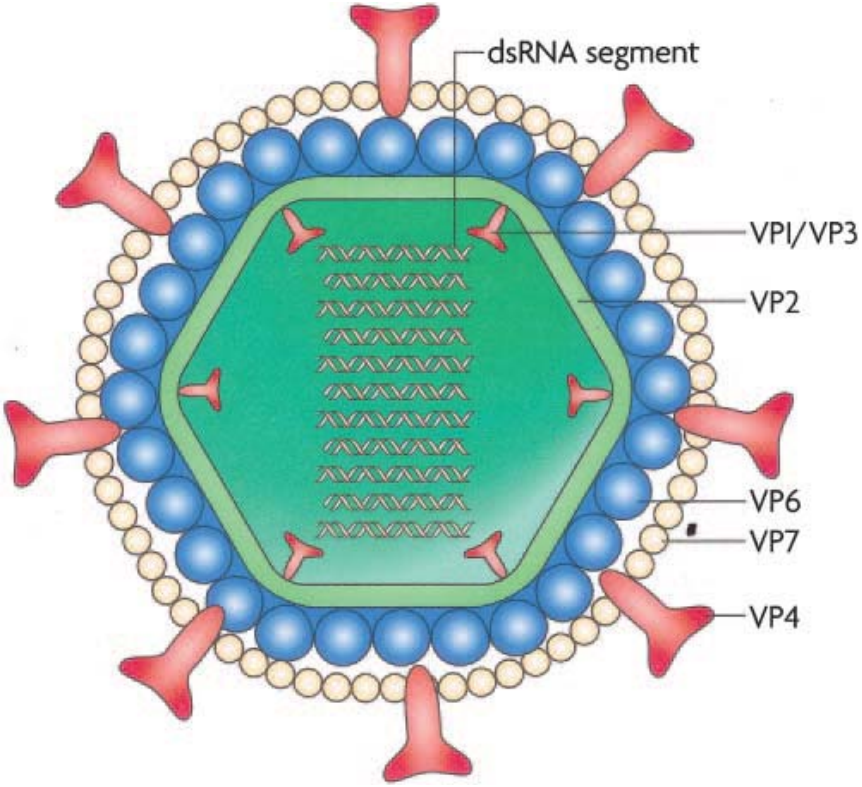
Almost all children are infected with rotavirus in early childhood. In developing countries, 65–80% of children have antibodies to rotavirus by 12 months

and 95% have been infected by 24 months. In general, children infected with rotaviruses during the first 3 months of life are asymptomatic, while those infected for the first time after the age of 3 months are usually symptomatic. Because natural infection confers some immunity to disease on subsequent exposures and because this protection increases with each subsequent exposure, the highest rates of rotavirus disease occur between 3 months and 2 years of age[101]. In many developing countries, however, the highest rates of illness occur among children aged 6–11 months. The incidence of rotavirus diarrhoea is similar among children in developed and developing countries. Attempts to control infection by improving water or food are therefore unlikely to alter the incidence of infection[12].Humans appear to be the main reservoir of rotavirus infections. The exact modes of transmission are unknown but are presumed to involve droplet or direct contact spread via the faecal-oral route.

Rotavirus disease burden[104]

Rotavirus infects nearly every child by the age of 3-5 years. The median age of a primary rotavirus infection is younger in developing countries, ranging from 6 to 9 months (80% occur among infants <1 year old). Developing countries often exhibit one or more periods of more intense rotavirus circulation against a background of year-round rotavirus transmission and a great diversity of rotavirus strains. Every year, rotavirus gastroenteritis is estimated to cause approximately 527,000 (475,000-580,000) deaths globally among children <5 years old. Most of these deaths occur in developing countries and 90% of the rotavirus-associated fatalities occur in Africa and Asia alone. Globally, >2 million children are hospitalized each year for rotavirus infections.

Structure ,genome and gene protein assignment:



The term ’rota’ (latin word) means a ‘wheel’. It is a double stranded RNA virus 65-70 nm in size, resembling little wheels with short spokes radiating from a wide hub to an outer rim.

Rota viruses have a genome of 11 segments of double stranded RNA. The RNA segments code for six structural proteins (VP1,VP2,VP3,VP4,VP7) and six nonstructural proteins(NSP1-NSP6).

The structural proteins make up a triple layered particle and are located in the core (inner layer;VP1-3),inner shell(intermediate layer;VP6) and outer shell (outer

layer;VP4,VP7). The wheel like appearance by electron microscopy of the triple layered particles is diagnostic.

The genome is compromised within the core shell formed by VP2. The transcription complex consisting of VP1 and VP3 is attached at the 12 five fold symmetry positions on the inside of the shell. The addition of VP6 (260 trimers) produces double layered particles, and further addition of VP7 (780 molecules) and VP4 (60 dimers) leads to formation of triple layered particles which are infectious virions after VP4 cleavage[21].

The triple layered capsid is ordered in five-, three-, and two fold symmetry axes and is perforated by 132 aqueous channels. The class I channels are necessary components to allow transcription in sub viral particles[110]

Genes, gene protein assignments, and functions of proteins of group A rota virus[21]

RNA segment		**Protein product**			**functions**
No.	Size (bp)	Description	location	No.of molecules Per virion	
1.	3302	VP1	Inner core	12	RNAdependentRNA polymerase;binds ssRNA;complex with VP3
2	2690	VP2	Core	120	Binds RNA ;required for replicase activity of VP1
3	2591	VP3	Inner core	12	Guanyltransferase,Methyl transferase;complex with VP1

4	2362	VP4	Outer capsid(dimer)	120	Hemagglutinin;cell attachment;neutralization antigen;fusogenic;protease enhanced infectivity
5	1611	NS53,NSP1 (VP5)	Nonstructural	Not applicable	Binds RNA;nonessential for replication
6	1356	VP6	Inner capsid(trimer)	780	Group and subgroup specific antigen;protection(?intracellular neutralization)required for transcription
7	1059	VP9(NSP3)	Nonstructural (dimer)	Not applicable	Binds RNA and eIF-4G1;inhibits host cell translation
8	1104	NS35,NSP2 (VP8)	Nonstructural (octamer)	Not applicable	Binds RNA; NTPase;helicase;involved in +strand RNA packaging
9	1062	VP7	Outercapsid (trimer)	780	Neutralization antigen;binds ca
10	751	VP12(NSP4)	Nonstructural	Not applicable	Intracellularreceptor,viral enterotoxin;protection by specific ab
11	667	VP11(NSP5) NSP6	Nonstructural Nonstructural	Not applicable	Binds RNA ;protein kinase;interacts with NSP2,VP2 AND NSP6 interacts with NSP5

CLASSIFICATION[110]:

1. According to serological cross reactivity of the inner capsid protein VP6,five groups (A-E) have been firmly established, and two more groups (F,G) are likely to exist. Within group A rota viruses, there are subgroups(I;II;I+II;nonI;nonII) according to exclusive reactivities with two VP6 specific monoclonal antibodies.

2. As the two surface proteins (VP4 andVP7) carry neutralization specific antigens,a dual classification scheme has been established. The system differentiates G types(VP7 specific,G for glycoprotein) and P types(VP4 specific,P for protease sensitive protein). So far 14 diferent G types and more than 20 P types have been detected indicating extensive genomic diversity within group A rota viruses.

The correlation of G serotypes and their genotypes is practically complete. For many P genotypes, no serotype has yet been established. Thus P serotype and genotype are designated separately but jointly, the latter in square brackets.

As VP4 and VP7 are coded by different RNA segments and as rota viruses of the same group reassort readily in doubly infected cells, the observed diversity due to combination of VP4 and VP7 is very large.

REPLICATION[21]:

Triple layered rota virus particles (infectious virions) attach to the surface of the host cell by its outer layer protein VP4 interacting with sialic acid or other receptors. After endocytosis,VP4 andVP7 are removed . The resulting double layered particles are transcriptionally active. The mRNAs are translated into proteins. Rota virus cores are formed consisting of VP2,VP1 and VP3 into which mRNA molecules are incorporated. Within the core, ssRNA segments are replicated to form dsRNA genome. Rota virus cores interact with VP6 to form double layered particles(DLPs) which in turn aggregate to form viroplasm. DLPs bud through rough endoplasmic reticulum facilitated by NSP4. In the RER, DLPs acquire VP4 and VP7 to form triple

layered particles(TLPs).The transiently developed envelope is shed before complete maturation. TLPs are released by cell lysis or by non classical vesicular transport.

PATHOGENESIS[110]:

1. Extensive cellular necrosis of the gut epithelium leads to villous atrophy, loss of digestive enzymes, a reduction of absorption and increased osmotic pressure in the gut lumen, resulting in the onset of diarrhoea.
2. This is followed by a reactive crypt cell hyperplasia accompanied by increased fluid secretion.
3. Viral factors: VP4,VP3,VP7 and NSP1,NSP2,NSP4 NSP4 as an enterotoxin increases intracellular calcium concentration disturbing the cellular homeostasis.

CLINICAL FEATURES: Incubation period:1-2 days

Sudden onset of watery diarrhoea lasting 4-7 days,vomiting and rapid dehydration. Rhinorrhoea, congestion of tympanic membrane and pharynx,perianal excoriation may be present. Depending upon the degree of dehydration, lethargy or even shock may be present.

NATURAL PROTECTION[17,79]

Most symptomatic rotavirus infections occur between 3 months and 2 years of age, with a peak incidence between 7 and 15 months. Rotavirus infections are more likely to be severe in children 3 to 24 months of age than in younger infants or older

children and adults . Although children can be infected with rotavirus several times during their lives, initial infection after 3 months of age is most likely to cause severe diarrhoea and dehydration.

GEOGRAPHICAL DISTRIBUTION OF ROTAVIRUS:

Rotavirus infection has been reported throughout the world. Studies between 1986 and 2006 showed that more than 51 rotavirus genotypes were found in Brazil. Approximately 43 of genotype was that of P[8]G1, followed by P[8]G9 and P[4]G2 . In Kenya, the genotype G1 was mainly observed up to the year 2002. Then G9 has emerged as the most predominant genotype and followed by a less frequent genotype G8 .Genotype P[8]G9 was mainly found throughout Latin America. In the United States, the G9 genotype was detected in a 1995–1996 outbreak [82]. In Australia, the overall G9 detection rate increased up to 29 % in 2001[48]. In Japan, G9 was mainly reported in 1998–1999 . In India, G9 strains were detected and were usually found in combination with the P[11] or P[6] genotypes at a detection rate of about 20 % .While genotypes G1P8, G2P4, G3P8, and G4P8 were also seen among Indian children (33 %), strains of P6 (G1P6, G2P6, G3P6, G4P6, and G9P6), which primarily infect asymptomatic newborns but are rare in children with diarrhea were common in India (43 %) [81]. The P[8]G9 was found in New Delhi in late 1998 . In Europe G1–G4 and G9 were the most prevalent genotypes identified: Genotype G1 was identified in Spain, Sweden, and the United Kingdom;G9 in Italy, France,and Belgium; and genotype G4 in Germany. Only the G4 and G9 genotypes[70] were identified in all areas.

DIAGNOSIS[58] OF ROTA VIRUS:

Rotavirus is shed in high concentration in the stool (~10^{11} particles/ml of faeces) of children with gastroenteritis. Therefore measurement of rotavirus antigen in the stool has been used to identify rotavirus infected patients

Rotavirus Detection:

Techniques for rotavirus detection include:

• Electron microscopy(EM)

• Antigen detection

1. Enzyme immunoassay (EIA)
2. Latex agglutination and lateral flow immunoassay (immunochromatography)
3. Nucleic acid detection (PAGE) and
4. Nucleic acid amplification (RT-PCR)

Electron microscopy

Electron microscopy is highly specific for detection of rotavirus and is as sensitive as some EIAs. However, the method is too labour intensive for routine detection of rotavirus in large numbers of stool specimens. In addition, EM requires an expensive instrument and highly trained personnel and cannot distinguish between rotaviruses of different groups.

Antigen detection

The most widely used methods for rotavirus diagnosis are based on detection of protein antigens on rotavirus particles in stool specimens. The most appropriate antigen detection format for large-scale surveillance studies is an EIA that uses

rotavirus specific antibodies to capture antigen onto wells of plastic plates. The antigen is then detected in a colorimetric reaction using a second rotavirus-specific antibody coupled to a detector enzyme. The EIA format is highly sensitive and specific and is adaptable to large sample volumes in the 96-well plate format. The optical density (OD) results can be easily recorded with a standard plate reader, permitting analysis of results with standard computer programs.

Latex agglutination, utilizing latex particles coated with anti-rotavirus antibodies can be used as an alternative to EIA and rapid near patient tests using immunochromatographic methods are being used widely in consulting rooms.

Nucleic acid detection

Because of the large quantities of rotavirus present in stool samples from children with gastroenteritis, the viral nucleic acid segments can be visualized directly after extraction from virus particles, by electrophoresis on acrylamide gels, and staining with ethidium bromide or silver nitrate. After electrophoresis, human rotavirus Groups A, B, and C have distinct patterns of gene-segment distribution, designated electropherotypes. The PAGE method has sometimes been used to diagnose Group A rotavirus infections for surveillance studies. However, this method is very labour intensive and time consuming.

A variety of sensitive conventional or real-time reverse-transcription polymerase chain reaction (RT-PCR) methods have been developed based on primers specific for several different rotavirus genes. These methods have been particularly useful in detecting rotavirus in extra-intestinal tissues, in studies of the duration of

viral shedding in stool and the correlation between disease severity and virus load. RT-PCR is also useful for verifying that RNA extracts contain intact rotavirus RNA. However, it is relatively expensive and labour intensive and detects low copy numbers of rotavirus.

Rotavirus Characterization:

Serotyping and subgrouping with monoclonal antibodies:

Enzyme immunoassays allow determination of rotavirus VP6 subgroup and VP7 serotype using serotype specific monoclonal antibodies. The five most common rotavirus G serotypes (G1, G2, G3, G4, G9) can be assigned a serotype directly from faecal material using several Enzyme Linked Immunosorbent Assay formats incorporating monoclonal antibodies (Mabs) that bind in a serotype-specific manner to the VP7 protein. Similarly, VP6 subgroupings I, II, I & II and non-I, II can be assigned using binding specificity of VP6 Mabs. Studies using serotyping Mabs have typically typed 60%-70% of strains circulating in the community. The method is rapid and inexpensive.

VP7 serotyping:

A disadvantage of Mab serotyping is that a substantial fraction of the rotaviruses in fecal specimens cannot be serotyped. However, for some collections of rotavirus specimens, a large percentage of samples will need to be analyzed by RT-PCR to determine the genotypes of the strains not typeable with Mabs. Another drawback of Mab serotyping is the need for continual supplies of Mabs and rotavirus hyperimmune antisera, both of which must be produced in animals.

VP4 serotyping: Many cross-reactive epitopes observed between different P serotypes/subtypes in rotavirus field isolates precludes the use of this assay for routine P typing studies.

VP6 subgrouping:

Polyclonal antibodies to the most abundant virion protein, VP6, are cross-reactive among all human and animal rotaviruses and largely define the group reactivity of rotaviruses. In contrast, some Mabs to the VP6 protein react specifically with different rotavirus strains.

Polyacrylamide gel electrophoresis (PAGE):

Rotavirus dsRNA can be detected in clinical specimens by extraction of the viral RNA and analysis by electrophoresis on a polyacrylamide gel followed by silver staining. Rotavirus dsRNA has 11 segments. During electrophoresis through the gel, these negatively charged macromolecules separate according to size. The patterns of dsRNA can be visualized in the gel by staining with silver nitrate .The dsRNA extracted from Group A rotaviruses can be split into four size classes: four large segments, two medium-sized segments, three small segments, and the two smallest segments. Group A human and animal rotaviruses also display two electropherotypes: "long" and "short. Although this relatively time-consuming method requires a trained technologist, the main advantage of PAGE is the lack of ambiguity in the results.

RNA extraction methods[58]:

Several methods have been described for the release and/or extraction of rotavirus RNA from clinical specimens. The purpose of these methods is to:

• Disrupt infected cells and microorganisms, resulting in the release of nucleic acids

• Protect the nucleic acids from degradation

• Remove inhibitors of amplification

• Concentrate the target nucleic acid

• Recover the nucleic acid in an environment suitable for its use in the PCR

Method involving crude lysis of microorganisms in specimens containing a high concentration of the target nucleic acid might be suitable for PCR, but additional nucleic acid extraction procedures are typically required to remove inhibitors and concentrate the target nucleic acid. Crude lysis might involve physical methods, such as freeze-thawing or sonication, or chemical methods using detergents, enzymes, or chaotropic agents. Some nucleic acid extraction methods rely on the differential solubilities of nucleic acids and proteins in phenol and water, as in phenol-chloroform extraction methods, or the ability of nucleic acids to bind to silica, as in silica guanidinium isothiocyanate extraction methods. Concentration of extracted nucleic acid is accomplished by precipitation with ethanol.

RT-PCR:

Rotaviruses in clinical specimens can be detected and G and P types determined by extraction of the viral RNA from faecal specimens and analysis by semi-nested RT-PCR with primers specific for regions of the genes encoding the VP7 (G-type) or VP4 (P-type). The objective is to obtain genotype-specific PCR products for analysis on an agarose gel or sequencing gel. RT-PCR of rotavirus dsRNA has three steps: 1) denaturation of dsRNA, 2) reverse transcription of dsRNA, and 3)

amplification of cDNA. PCR consists of these steps: 1) heating the DNA to be amplified to separate the two template strands, 2) adding two primers that are complimentary to the region to be amplified, 3) adding a heat-stable DNA polymerase enzyme that catalyses the extension of the primers using the DNA strand as template, and 4) repeating the cycle, with the newly synthesised cDNA heat-denatured and the enzymes extending the primers attached to the liberated single DNA strands.

RT-PCR genotyping:

RT-PCR genotyping methods are used increasingly as a surrogate for serotyping. Since RT-PCR genotyping can determine both G and P types, confirm results, and characterize non-typeable strains with nucleotide sequencing, RT-PCR genotyping is the method of choice for most laboratories.

Rotavirus genotyping methods are based on semi-nested RT-PCR, in which viral RNA extracted from fecal specimens is reverse-transcribed and amplified by PCR in the presence of consensus primers for the rotavirus genes specifying G (gene 9) or P (gene 4) serotype.

The primers are selected to be homologous to strains from different serotypes, so that one primer pair can be used to amplify most human rotavirus strains. The DNAs from the first amplification cycle are used as a template in a second PCR in the presence of one of the original consensus primers and a mixture of genotype-specific primers of opposite polarity from the consensus primer, each designed to yield a product of different size.

Issues in strain genotyping:

1.Non typeable(NT) strains resulting from genetic variation in common strains.

2.Non typeable strains resulting from the presence of novel strains..

Examples include the detection of types G5, G6, G10, G12, P[11], and P[14] among NT strains. Although these novel strains have usually been detected at very low frequency, examples of high-incidence detection of such strains have also been reported.

Other reasons for an inability to type strains:

False-positive EIA, insufficient or degraded RNA, the presence of residual stool inhibitors in the RNA extract, or technical problems with the assay itself.

Confirmation of results:

Although genotyping methods have been shown to be >90% accurate, misidentification by RT-PCR methods does occur. Although several confirmation methods have been described (e.g., Southern hybridization with cDNA and oligonucleotide probes or serotyping methods), sequence analysis has become the standard for both confirmation and identification of NT strains.

Other characterization methods:

Additional characterization techniques include cultivation in cell culture to amplify the amount of virus present and dilute out stool inhibitors, followed by repetition of routine methods or sequencing. If the sequences of the VP4 and/or VP7 genes suggest a novel serotype, it might be necessary to prepare hyperimmune sera to

the strains and conduct cross-neutralization tests to determine if the strains are antigenically distinct from known rotavirus serotypes. These types of studies have helped demonstrate that some rare human rotaviruses arose through interspecies transmission of an animal rotavirus to humans. Such studies also suggest that some strains, both common and uncommon, probably arose through reassortment between human and animal rotaviruses Thus, the analysis of untypeable rotavirus strains from surveillance studies has been important in defining the genetic diversity and possible origin of many human rotaviruses.

Rotavirus vaccines:

Currently Licensed Vaccines:

1. Human-Bovine Rotavirus Reassortant Vaccine (RotaTeq)
2. Live-Attenuated Human Rotavirus Vaccine (Rotarix)

Vaccine	Parent strain	genotype Formulation	Dose regimen
RotaTeq	Bovine rotavirus strain WC3, P7[5]G6	5 reassortants; 4 reassortants with the VP7gene from G1, G2, G3, or G4 and 1 reassortant with the VP4 P1A[8] gene from the human rotavirus parent strain with the remainder of the genes from the WC3 bovine rotavirus Parent strain	3 oral doses at 2, 4, and 6 months of age
Rotarix	Human rotavirus strain 89-12, P1A[8]G1	No reassortants; RIX4414, a further-passaged human rotavirus 89-12 strain	2 oral doses at 2 and 4 months of age

BACTERIAL AGENTS:

ESCHERICHIA COLI:

Escherichia coli was first identified by Theodor Escherich, German paediatrician while studying on the intestinal flora of infants. He described the organism in 1885 as bacterium coli commune and established its pathogenic properties in extra intestinal infection.

Diarrhoeagenic E.coli is classified as[65]

1. Enterotoxigenic E.coli (ETEC)
2. Enteropathogenic E.coli (EPEC)
3. Enterohaemorrhagic E.coli (EHEC)
4. Enteroinvasive E.coli (EIEC)
5. Enteroaggregative E.coli (EAEC) and
6. Diffusely Adherent E.coli (DAEC)

ETEC and EPEC are important causes of diarrhoea in infants and ETEC is a primary cause of traveller's diarrhoea. Diarrhoeagenic E.coli can produce watery,cholera like diarrhoea (ETEC),dysentery like symptoms(EIEC),and grossly bloody diarrhoea(EHEC). E.coli O157:H7,one of the common EHEC serotypes was first described by Karmali et al in 1982. It produces shiga like toxin and is associated with haemolytic uraemic syndrome,especially in children[77]. The medium of choice for this organism is Sorbitol Mac conkey agar. Other tests for detection are MUG (4 methyl umbelliferyl beta D Glucuronide)test, latex agglutination test and PCR for detecting genes stx1 and stx2[49].

SHIGELLA:

Shigella dysenteriae ,one of the four species of Shigella ,first was isolated by Shiga in 1898.There are four species-S,dysenteriae,S.flexneri,S.boydii and S.sonnei. S.flexneri and S.dysenteriae are the predominant species in developing countries. Endemic Shigellosis is typically a paediatric disease with most patients below 5 years of age.

SALMONELLA:

Age specific attack rates for Salmonella infection are highest in children younger than 4 years of age with higher invasiveness and mortality in infants. Antimicrobial treatment is not indicated for patients with uncomplicated, noninvasive salmonella gastroenteritis because therapy may prolong the the duration of carriage.

VIBRIO CHOLERAE:

Vibrio cholerae is most dangerous vital pathogen which has morbidity and mortality rate higher than other organisms.

PARASITIC AGENTS:

Regarding parasitic causes, Entamoeba histolytica is common in developing countries. Cryptosporidium has been documented in atleast of 5% of diarrhoeal stools in developing countries[16]. Giardia has bimodal age distribution with children less than 5 years most frequently affected.

TREATMENT:

The broad principles of management of acute gastroenteritis in children include oral rehydration therapy, enteral feeding and diet selection, Zinc supplementation and additional therapies such as probiotics.

PREVENTION[66]

Preventive strategies includes

1. Promotion of exclusive breast feeding
2. Improved complimentary feeding practices like Vitamin A supplementation reduces childhood diarrhoea by 34%.
3. Improved water and sanitary facilities and promotion of personal and domestic hygeine.
4. Behavioural change strategies through hand washing promotion and access to soap reduce the burden of diarrhoea in developing countries.
5. Rotavirus and measles vaccinations.

Materials And Methods

MATERIALS AND METHODS

TYPE OF STUDY: Cross sectional study

PERIOD OF STUDY: Oct 2011-Nov 2012

PLACE OF STUDY:

This study was conducted at the department of Microbiology, Stanley Medical College, Chennai. Samples were collected from Institute of Child health, Egmore Chennai-3 and Institute of social paediatrics, Stanley, Chennai-1 .

SELECTION OF STUDY GROUP

A total of 150 children, 0-5 years of age were included in the study. The patients were clinically examined and selected for the study after obtaining the history.

INCLUSION CRITERIA:

Children less than 5 years of age with complaints of more than 3 episodes of watery stools with or without mucus and blood for a period less than 2 weeks and not on antibiotics or any laxatives were included in the study.

EXCLUSION CRITERIA:

Children above 5 years of age, Children with complaints of diarrhoea more than 2 weeks and children who acquired diarrhoeal illness after hospital admission were excluded from the study.

SELECTION OF CONTROL GROUP:

A total of 50 children aged 5 years or below admitted to the hospital for causes other than diarrhoea were included as controls.

ETHICAL CONSIDERATION

Ethical and research clearance was obtained from the Ethical committee,Stanley Medical College. Permission to conduct the study was sought from the respective hospital authorities. Informed consent was obtained from parents/ guardians of the child before enrolment into the study.

STATISTICAL ANALYSIS:

The matching of study and control group was analysed by students 't' test for independent variables and the other variables were analysed by chi square test. The 'z' test of proportions between the groups was performed wherever necessary. The above statistical procedures were performed by the statistical package PASW(Predictive and Analysis Software)statistics -18 so called SPSS. The p values less than 0.05 were considered as significant.

COLLECTION AND TRANSPORT OF STOOL SAMPLES:

The stool samples were collected in a clean wide mouthed sterile container, labelled properly, transported to the laboratory in ice pack and processed within one to two hours.

MICROBIOLOGICAL ANALYSIS:

A portion of each sample was stored in screw capped vials at -70ºc for analysis of rota virus.

Macroscopic examination:

The stool samples were examined for colour, consistency, presence of blood, mucus, segments of parasite or worms.

Direct microscopic examination:

Saline and Iodine wet preparations were examined for the presence of erythrocytes, leukocytes,trophozoites, ova and cysts of the parasites.

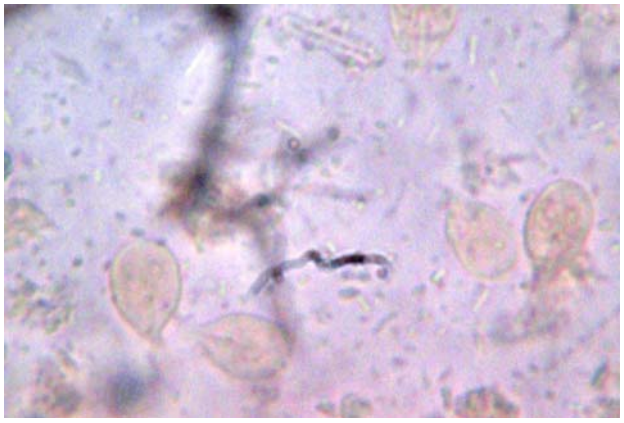
Saline wet mount[41]:

A minute portion of the faeces is diluted with 0.9% NaCl and a drop of it was placed on a clean glass slide. A 22×22 mm clean coverslip was carefully placed over the suspension avoiding air bubbles.

Iodine wet mount[41]:

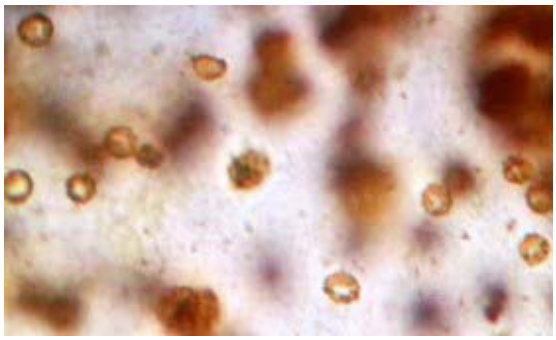
A drop of diluted Lugol's iodine is added to the drop of saline emulsion of faeces on a clean glass slide. Cover slip is put on . Both the preparations were observed under low power objective and suspicious object was examined under high power objective.

Saline mount showing trophozoites of Giardia lamblia

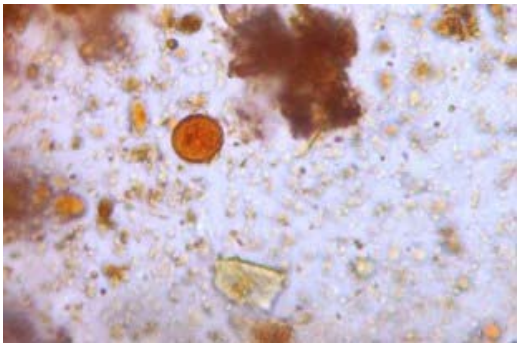


Iodine mount showing red blood cells and leukocytes

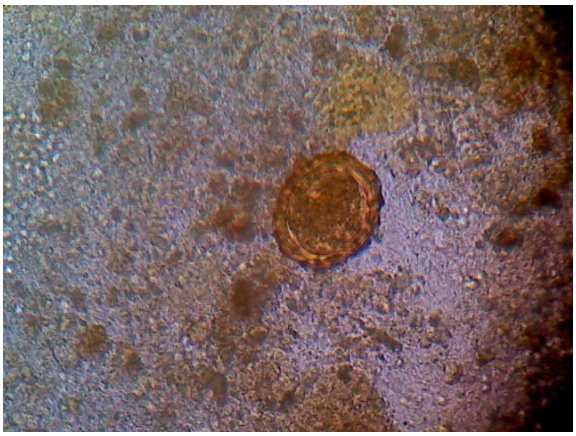
Iodine mount showing red blood cells



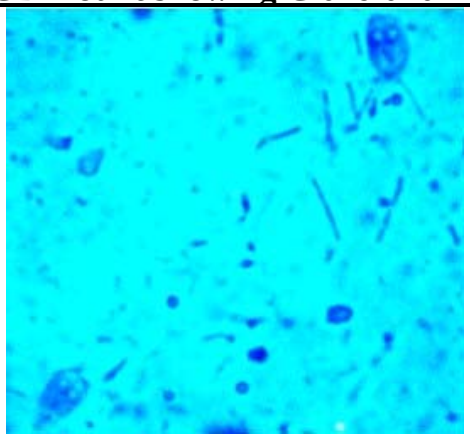
Iodine mount showing cyst of Entamoeba histolytica



Iodine mount showing fertilised egg of Ascaris lumbricoides



LPCB mount showing Giardia lamblia



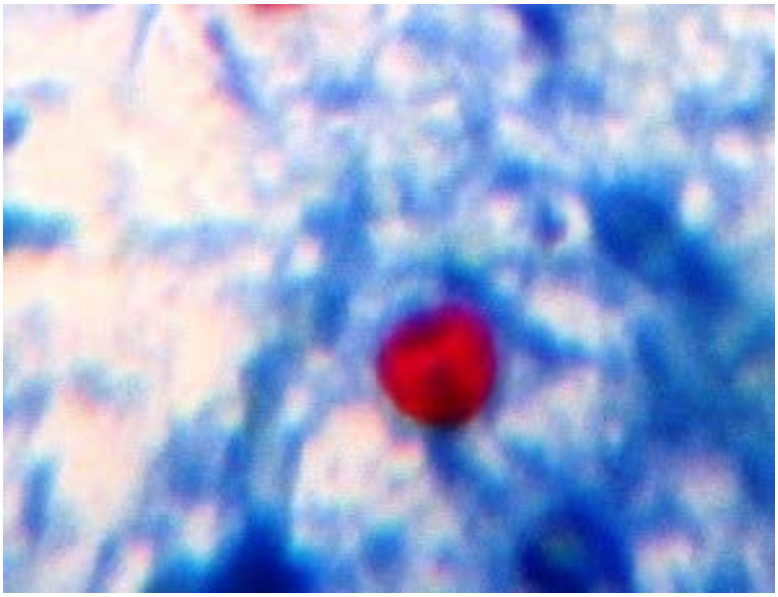
Modified Kinyouns's Acid Fast staining method was used for detecting coccidian parasites in the stool specimen[54].

1. Heat fixed faecal smear was covered with a strip of a filter paper. The slide was flooded with Kinyoun's carbol fuchsin and allowed to stain for 5 min.
2. The stain was tipped off and filter paper removed. The slide was rinsed briefly(3-5 sec) with 50% ethanol and then thoroughly with water.
3. 1% sulphuric acid was used to decolorize for 2 min or until no more colour ran from the slide. Slide was rinsed in water and drained.
4. Counterstaining was done with methylene blue for 1 min. Slide was rinsed with water and air dried.
5. The slide was examined under oil immersion objective.

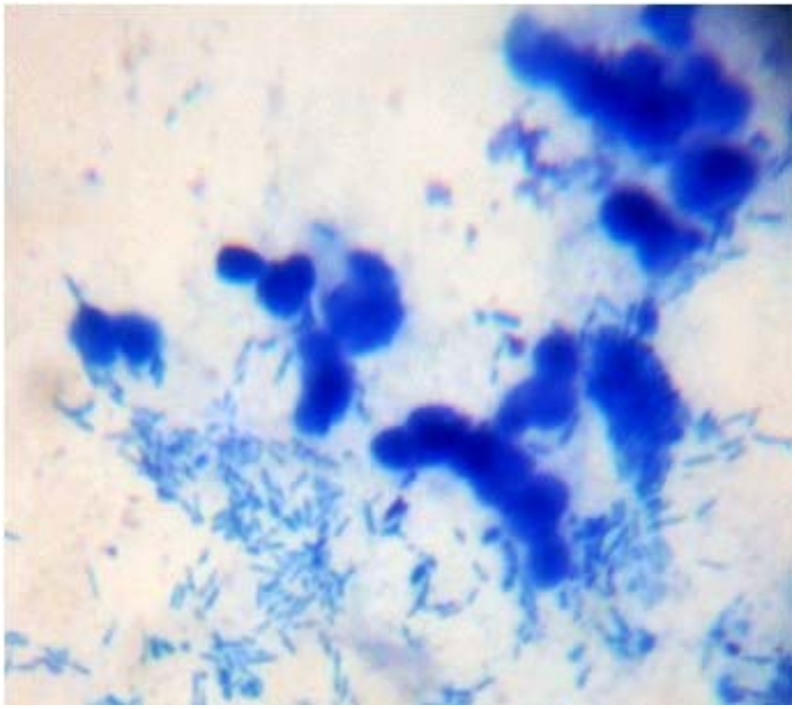
Culture:

Samples were cultured on Mac conkey agar, Sorbitol MacConkey agar, Xylose lysine deoxycholate agar and Thiosulphate citrate bile salts sucrose (TCBS) agar and incubated at 37□C overnight. Samples were also inoculated in selenite F broth and alkaline peptone water, incubated for 18 and 4 h, respectively, for enrichment of Salmonella and Vibrio spp. and subcultured on xylose lysine deoxycholate agar and TCBS agar, respectively. If Vibrio cholerae was suspected, motility was observed by hanging drop method. From each sample the lactose positive and lactose negative colonies were subjected to identification procedures. Shigella, Salmonella and Vibrio cholerae isolates were serotyped by slide agglutination test using specific antisera.

Modified Acid Fast stain showing Cryptosporidium parvum

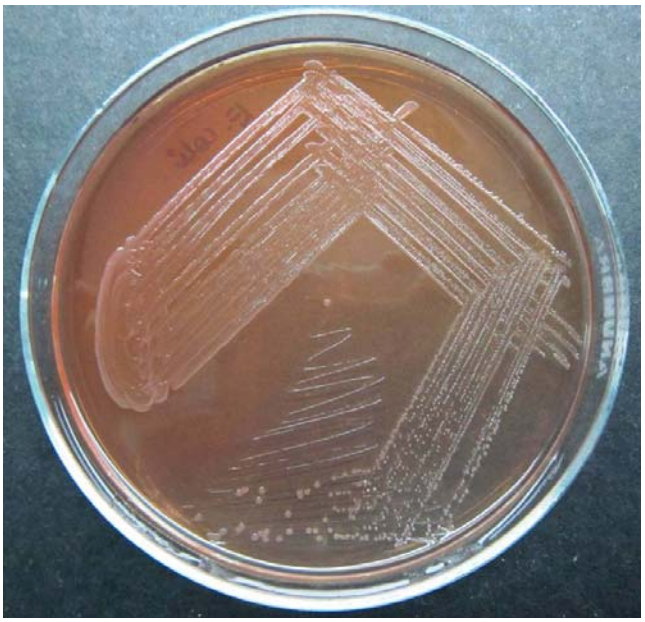


Modified Acid Fast stain showing pus cells



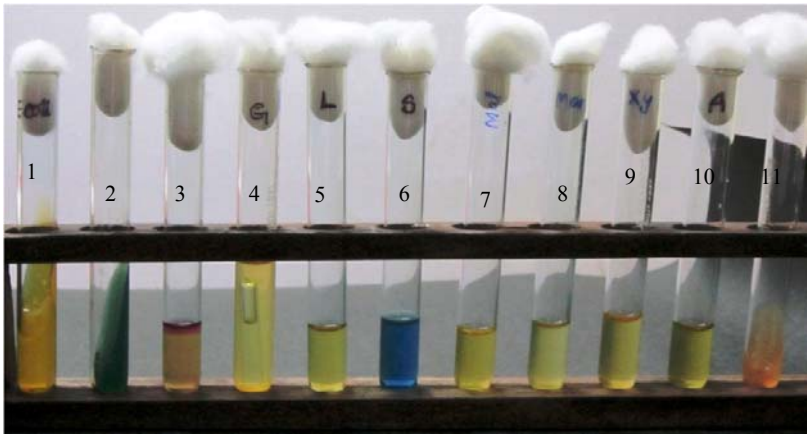
Mac Conkey agar showing lactose fermenting colonies of Enterohaemorrhagic Escherichia coli

Sorbitol Mac Conkey agar showing colonies of Enterohaemorrhagic Escherichia coli



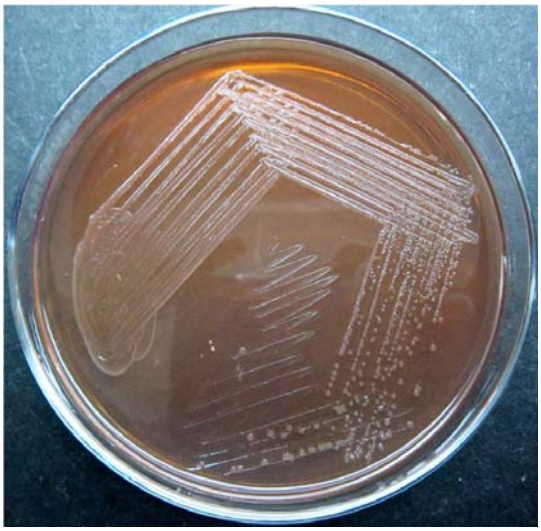
XLD plate showing colonies of Escherichia coli

Biochemical reactions of Escherichia coli



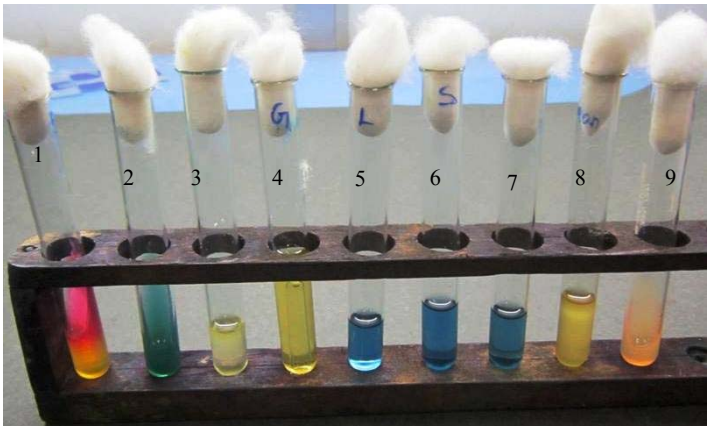
1. TSI-A/A
2. CITRATE-NOT UTILISED
3. INDOLE-POSITIVE
4. GLUCOSE- FERMENTED WITH ACID AND GAS
5. LACTOSE- FERMENTED
6. SUCROSE-NOT FERMENTED
7. MALTOSE-FERMENTED
8. MANNITOL- FERMENTED
9. XYLOSE - FERMENTED
10. ARABINOSE- FERMENTED
11. UREASE NEGATIVE

Mac Conkey agar showing non lactose fermenting coloniesof Shigella



XLD plate showing colonies of Shigella

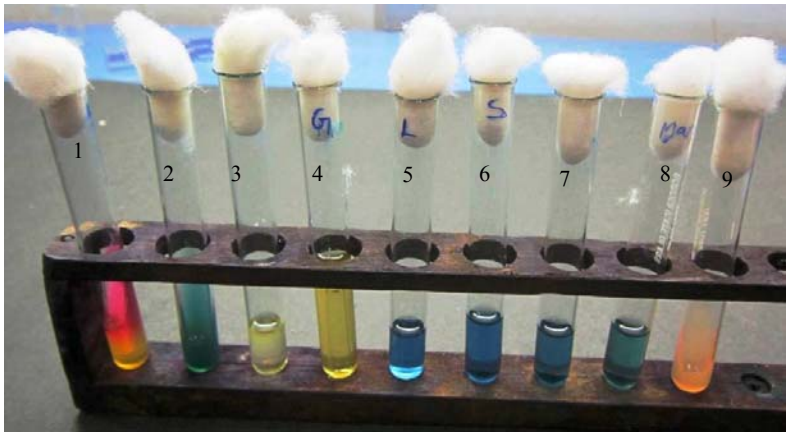
Biochemical reactions of Shigella flexneri



1. TSI-K/A
2. CITRATE-NOT UTILISED
3. INDOLE-NEGATIVE
4. GLUCOSE- FERMENTED WITH ACID
5. LACTOSE- NOT FERMENTED
6. SUCROSE-NOT FERMENTED
7. MALTOSE- NOT FERMENTED
8. MANNITOL- FERMENTED
9. UREASE NEGATIVE

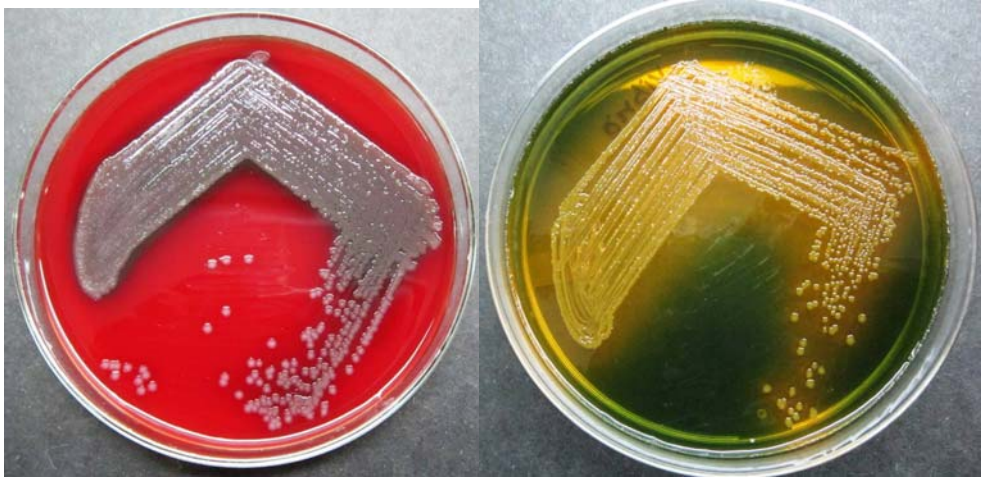
Slide Agglutination of Shigella flexneri

Biochemical reactions of Shigella dysenteriae

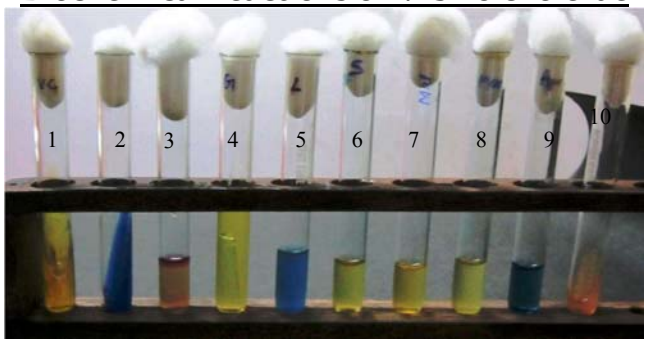


1. TSI-K/A
2. CITRATE-NOT UTILISED
3. INDOLE-NEGATIVE
4. GLUCOSE- FERMENTED WITH ACID
5. LACTOSE- NOT FERMENTED
6. SUCROSE-NOT FERMENTED
7. MALTOSE- NOT FERMENTED
8. MANNITOL- NOT FERMENTED
9. UREASE NEGATIVE

Blood agar plate and TCBS plate showing colonies of Vibrio cholerae

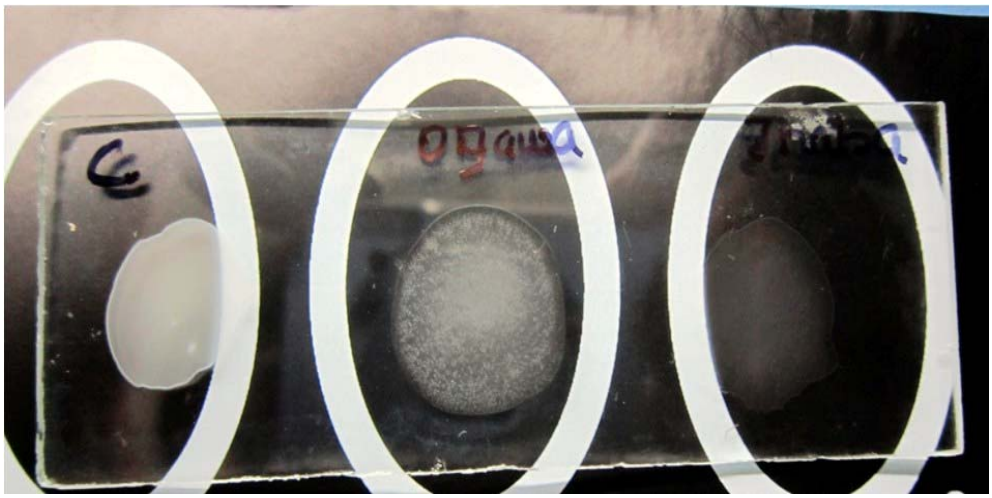


Biochemical reactions of Vibrio cholerae

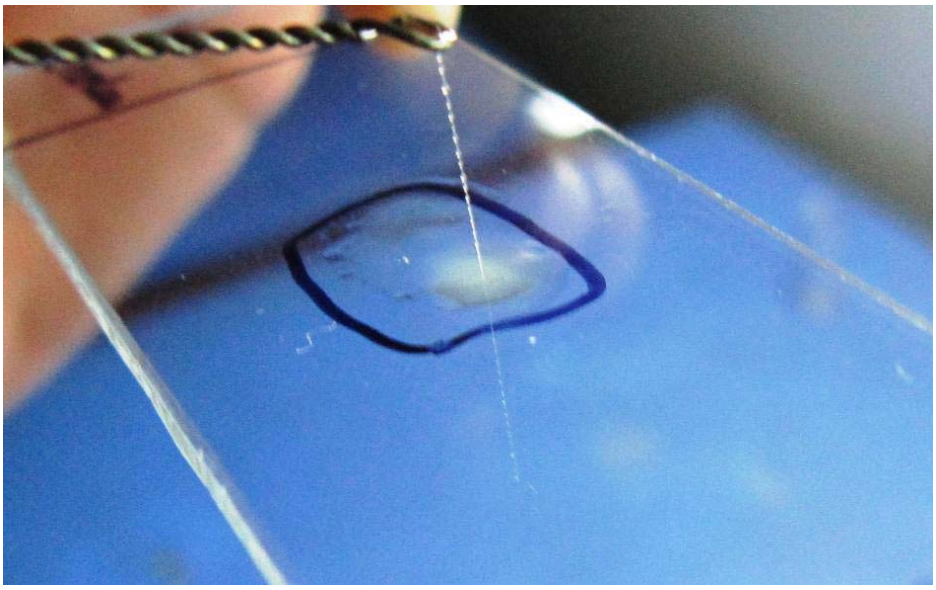


1. TSI-A/A
2. CITRATE- UTILISED
3. INDOLE-POSITIVE
4. GLUCOSE- FERMENTED WITH ACID
5. LACTOSE- NOT FERMENTED
6. SUCROSE- FERMENTED
7. MALTOSE-FERMENTED
8. MANNITOL- FERMENTED
9. ARABINOSE- NOT FERMENTED
10. UREASE NEGATIVE

Slide Agglutination of Vibrio cholera



String test for Vibrio cholerae



Similar to study group, the stool samples from control children were also processed by standard microbiological procedures.

The following tests were used for identification :

Catalase, Oxidase, Indole, Triple Sugar Iron agar, Citrate, Urease,carbohydrate fermentation tests and motility.

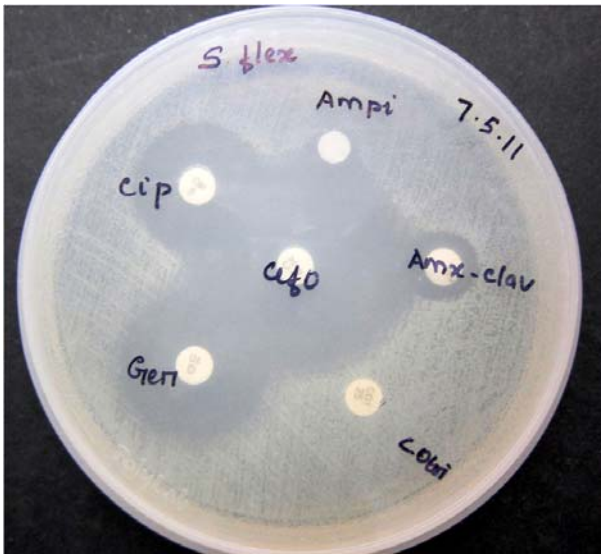
Antimicrobial susceptibility:

The significant isolates were subsequently tested for antimicrobial susceptibility by Kirby-Bauer disc diffusion method with antibiotics ampicillin 10 µg ,cefotaxime 30 µg, ciprofloxacin 5 µg,co-trimoxazole T1.25/S23.75 µg, gentamicin10 µg,amoxy clavulanic 20/10 µg acid, tetracycline30 µg and chloramphenicol 30 µg.

Mueller-Hinton agar plate was inoculated with 0.5 McFarland standard inoculums to obtain a lawn culture. Using sterile forceps, discs were placed over the agar surface, incubated at 37˚C for overnight. The results were interpreted as per Clinical Laboratory Standards Institute (CLSI) standards.

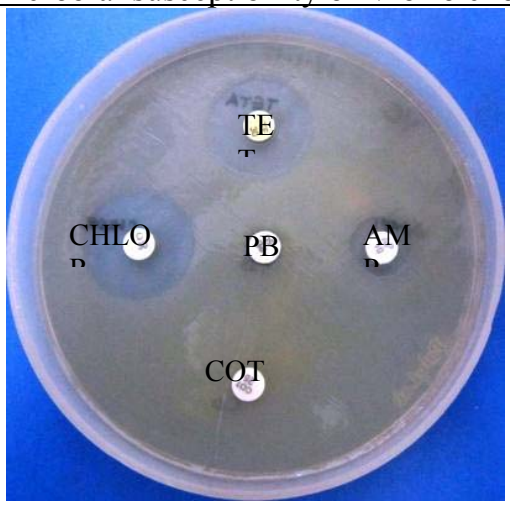
The Escherichia coli strain ATCC 25922 was included as a quality control in all tests.

Antimicrobial susceptibility of Shigella flexneri



AMPI- AMPICILLIN, CIP- CIPROFLOXACIN, CEFO- CEFOTAXIME, AMX-CLAV- AMOXY CLAVULANIC ACID, GEN- GENTAMYCIN, COTRI- COTRIMOXAZOLE

Antimicrobial susceptibility of Vibrio cholerae



TET-TETRACYCLINE,CHLOR-CHLORAMPHENICOL,PB-POLYMYXIN, AMP-AMPICILLIN, COT-COTRIMOXAZOLE

DETECTION AND CHARACTERIZATION OF ROTA VIRUS:

Detection of Rota virus:

Rota virus was identified from all stool samples with commercially available ELISA kit detecting group specific VP6 antigen for rota virus.

1. Antigen detection: was done by Enzyme Linked Immunosorbent assay method (ELISA) according to manufacturer's instruction.[RIDASCREEN]

Test principle

This is a sandwich-type of ELISA. Monoclonal antibodies against a capsid protein of gene 6 (VP6) of the rotaviruses are applied to the surface of the well in the microwell plate. This is a group-specific antigen which occurs with all rotaviruses which are pathogenic to humans. A suspension of the stool sample which is to be tested together with other monoclonal anti-rotavirus antibodies, which are conjugated with horseradish peroxidase, is pipetted into the well in the microwell plate for incubation.

ELISA kit for rota virus antigen detection

ELISA reader

ELISA washer

In the presence of rotaviruses, a sandwich complex forms which is made up of the immobilised antibodies, the unattached enzyme-labelled antibodies are removed during a further washing phase. After adding the substrate, the attached enzyme changes the colour of the previously colourless solution in the wells of the microwell plate to blue if the test is positive. On adding the stop reagent, the colour changes from blue to yellow. The extinction is proportional to the concentration of rotavirus antigens present in the sample.

Preparing the wash buffer:

1 part wash buffer concentrate was mixed with 9 parts distilled water

Preparing the samples:

Approx. 100 µl of sample was suspended in 1 ml sample dilution buffer in a labelled test tube and homogenised by mixing in a vortex mixer. Then centrifuged at 5000 rpm for 5 minutes. The supernatant was used in the test.

Procedure:

All reagents were brought to room temperature.

1. 100 µl of sample-dilution buffer (= negative control) was added to well A1 and B1.
2. 100 µl of positive control was added to wells c1 and D1.
3. 100 µl of the stool suspension added in the wells starting from E1.

4. Next, 2 drops (100 µl) of the enzyme-conjugated antibody [Conjugate] was added and, after mixing thoroughly, incubated at room temperature (20 – 25 °C) for 60 minutes.
5. After incubation the wells were washed 5 times using 300 µl wash buffer each time.
6. 2 drops (100 µl) of substrate was added to each well. Then incubated the plate at room temperature (20 - 25 °C) for 15 minutes in the dark.
7. After this, 1 drop (50 µl) stop reagent was added to each well to stop the reaction
8. After mixing carefully (by lightly tapping the side of the plate) absorbance was read at 450 nm using a reference wavelength ≥ 600 nm

Test validity:

Negative control must be less than or equal to 0.2

Positive control must be more than or equal to 0.8

Evaluation and interpretation:

Calculating the cut-off:

Cut-off = extinction for the negative control + 0.15

Test result:

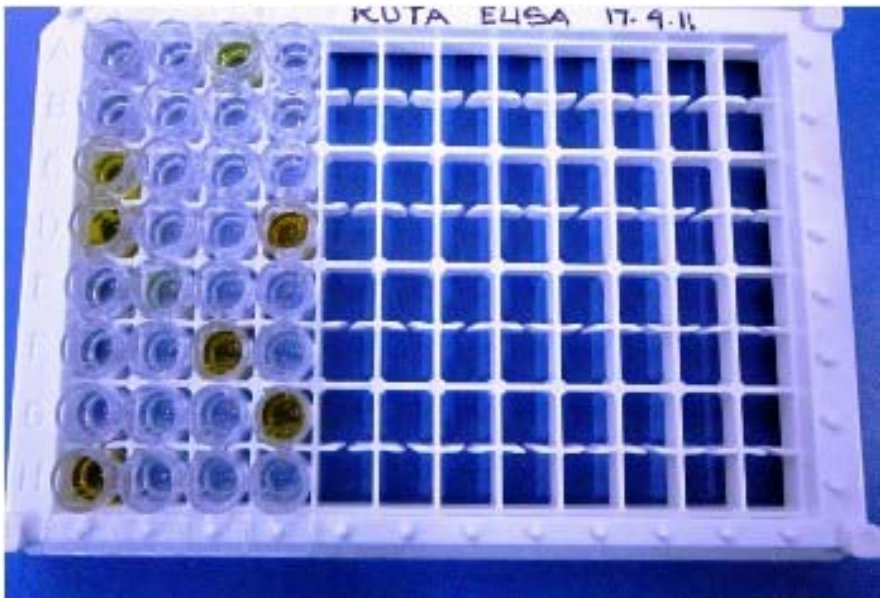
Samples are considered **positive** if their extinction is more than 10 % above the calculated cut-off.

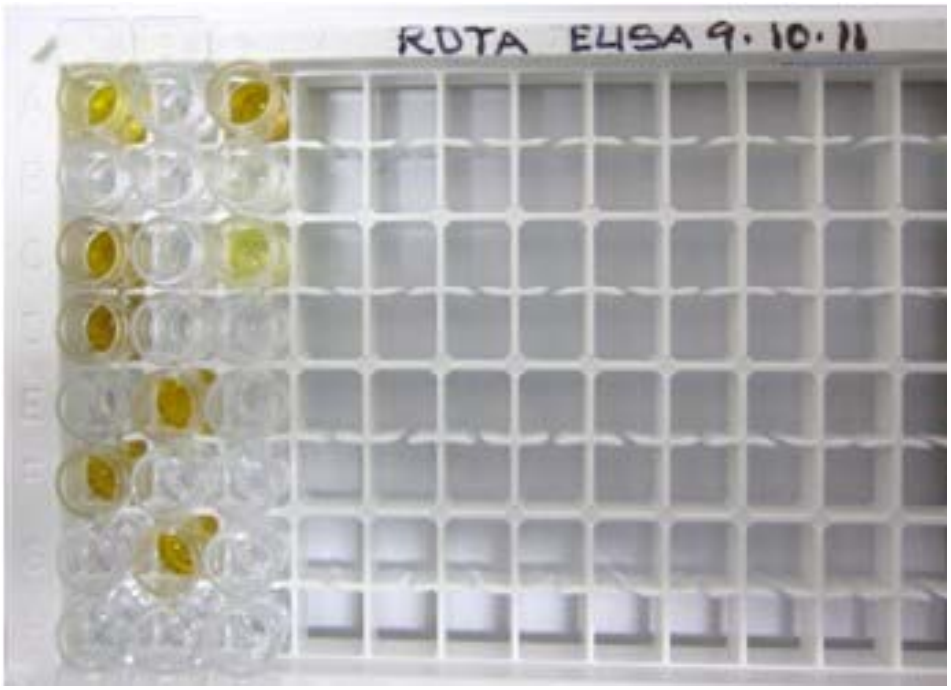
Samples are considered **equivocal** and must be repeated if their extinction is within ± 10 % of the cut-off. If repeating the test with a fresh stool sample again yields a value in the grey range, the sample must be considered negative.

Samples with extinctions more than 10 % below the calculated cut-off must be considered negative.

ELISA positive samples were subjected for characterization by RNA extraction and Reverse transcriptase Polymerase Chain Reaction as standardised by Invitrogen .

ELISA for rota virus antigen detection





Stanley Medical College

Plate Name :ROTAVIRUS
Tech ID :K.SUBHA
Filter :450/655 nm
CutOff :0.160

[redacted] 00:04:18
Kit.Batch :17.9.11
Plate Status :OK : MeanBlank = Nil
Cutoff Formula:NCtl+0.15

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.010 **NCtl**	0.007 **Neg**	0.603 **Pos**	0.004 **Neg**								
B	0.010 **NCtl**	0.006 **Neg**	0.005 **Neg**	0.042 **Neg**								
C	2.050 **PCtl**	0.006 **Neg**	0.005 **Neg**	0.005 **Neg**								
D	2.110 **PCtl**	0.005 **Neg**	0.007 **Neg**	2.389 **Pos**								
E	0.010 **Neg**	0.152 **Neg**	0.007 **Neg**	0.007 **Neg**								
F	0.010 **Neg**	0.009 **Neg**	2.362 **Pos**	0.011 **Neg**								
G	0.010 **Neg**	0.006 **Neg**	0.009 **Neg**	2.304 **Pos**								
H	2.390 **Pos**	0.004 **Neg**	0.006 **Neg**	0.006 **Neg**								

Validation :

NC < 0.2

Pc ≥ 0.8

Stanley Medical College

Plate Name :Rota virus
Tech ID :K Subha
Filter :450/655 nm
CutOff :0.160

09.10.11 13:38:33
Kit.Batch :09-10-11
Plate Status :OK : MeanBlank = Nil
Cutoff Formula:NCtl+0.15

1	2	3	4	5	6	7	8	9	10	11	12
1.760 **PCtl**	0.006 **Neg**	2.403 **Pos**									
0.010 **NCtl**	0.006 **Neg**	0.051 **Neg**									
2.320 **Pos**	0.005 **Neg**	0.387 **Pos**									
2.350 **Pos**	0.008 **Neg**	0.006 **Neg**									
0.010 **Neg**	2.300 **Pos**	0.007 **Neg**									
2.330 **Pos**	0.005 **Neg**	0.003 **Neg**									
0.000 **Neg**	2.277 **Pos**	-0.002 **Neg**									
0.000 **Neg**	0.001 **Neg**	0.002 **Neg**									

Validation : NC ≤ 0.2

PC ≥ 0.8

RNA EXTRACTION AND REVERSE TRANSCRIPTION:

RNA extraction was done by QIAmp viral RNA extraction method.

1. 560µl of prepared Buffer AVL containing carrier RNA was pipetted into a 1.5 ml microcentrifuge tube.
2. 140 µl of sample was added to the Buffer AVL in the microcentrifuge tube and mixed by pulse vortexing for 15 s.
3. Incubated at room temperature for 10 min.
4. The tube was centrifuged briefly to remove drops from the inside of the lid.
5. 560µl of ethanol (96-100%) was added to the sample and mixed by pulse vortexing for 15 s. Then centrifuged briefly to remove drops from the inside of the lid.
6. 630 µl of the solution from step 5 was applied to the QIAmp Mini column (in 2 ml collection tube) without wetting the rim.The cap was closed and centrifuged at 8000 rpm for 1 min. The QIAmp mini column was placed into a clean 2 ml collection tube and the tube containing filtrate was discarded.
7. The QIAmp mini column was opened and step 6 was repeated.
8. The QIAmp mini column was opened and 500µl of Buffer AW1was added. Then the cap was closed and centrifuged at 8000 rpm for 1 min. The QIAmp mini column was placed into a clean 2 ml collection tube and the tube containing filtrate was discarded.
9. The QIAmp mini column was opened and 500µl of Buffer AW2 was added. Then the cap was closed and centrifuged at 14000 rpm for 3 min. The QIAmp mini column was placed into a clean 2 ml collection tube and the tube containing filtrate was discarded. Then centrifuged at full speed for 1 min.
10. The QIAmp mini column was placed in a clean 1.5 ml microcentrifuge tube and the old collection tube with filtrate was discarded. The QIAmp mini

column was opened and 60µl of Buffer AVE was added for elution of RNA. Then the cap was closed and incubated at room temperature for 1 min and centrifuged at 8000 rpm for 1 min.

Thermo cycler

Agarose gel electrophoresis

Gel documentation system

Denaturation of ds RNA:

40 µl of extracted nucleic acid was added to 0.2 ml PCR tube and ds RNA was denatured at 97□c for 5 min and chilling the tube on ice for 2 min.

POLYMERASE CHAIN REACTION:

G Typing PCR (VP7):

1x mix (Full length gene , 1 st round):

2x buffer(with dNTPs and $Mgcl_2$)	12.5µl
Primer VP7 F(20pmoles/ µl)	1 µl
Primer VP7 R(20pmoles/ µl)	1 µl
Enzyme mix (Taq polymerase and Reverse transcriptase)	0.5 µl
RNase free water	5 µl

1. prepared 1st round PCR mix
2. 25 µl of PCR mix was added to each tube.
3. 5 µl of denatured RNA was added . For negative control RNase free water 5 µl was added.
4. The tubes were transferred to the PCR machine room.
5. Brief spin was done in microcentrifuge
6. The tubes were added to the thermocycler
7. For reverse transcription ,the temperature was set to 50ºc for 30 min.

8.For PCR, the following cycling conditions were set.

94 ºc	2 min	x1
94 ºc	1 min	
52 ºc	1 min	x35
72 ºc	1 min	
72 ºc	7 min	x1
15 ºc	hold	

Genotyping: 2nd round multiplex PCR:

For 1 x:

2x buffer(with dNTPs and $Mgcl_2$)	12.5µl
Primer VP7 R(20pmoles/ µl)	1 µl
Primer G1(20pmoles/ µl)	1 µl
Primer G2(20pmoles/ µl)	1 µl
Primer G3(20pmoles/ µl)	1 µl
Primer G4(20pmoles/ µl)	1 µl
Primer G8(20pmoles/ µl)	1 µl
Primer G9(20pmoles/ µl)	1 µl
Primer G10(20pmoles/ µl)	1 µl
Enzyme mix (Taq polymerase and Reverse transcriptase)	1 µl
RNase free water	18.5 µl

1. 2nd round PCR mix awas prepared.
2. 40 µl of PCR mix was added to each tube.

3. 10 µl of 1st round product was added. For negative control RNase free water 10 µl was added
4. The tubes were transferred to the PCR machine room.
5. Brief spin was done in microcentrifuge
6. The tubes were added to the thermocycler
7.. For PCR, the following cycling conditions were set.

94 ºc	4 min	x1
94 ºc	1 min	
42 ºc	2 min	x30
72 ºc	1 min	
72 ºc	7 min	x1
15 ºc	hold	

P Typing PCR (VP4):

1x mix (Full length gene , 1 st round):

2x buffer(with dNTPs and $Mgcl_2$)	12.5µl
Primer Con3 (20pmoles/ µl)	1 µl
Primer Con2 (20pmoles/ µl)	1 µl
Enzyme mix (Taq polymerase and Reverse transcriptase)	0.5 µl
RNase free water	5 µl

1. prepared 1st round PCR mix
2. 25 µl of PCR mix was added to each tube.

3. 5 µl of denatured RNA was added. For negative control RNase free water 5 µl was added
4. The tubes were transferred to the PCR machine room.
5. Brief spin was done in microcentrifuge.
6. The tubes were added to the thermocycler.
7. For reverse transcription ,the temperature was set to 50ºc for 30 min.
8. For PCR, the following cycling conditions were set.

94 ºc	2 min	x1
94 ºc 50 ºc 72 ºc	1 min 1 min 1 min	x35
72 ºc 15 ºc	7 min hold	x1

P Genotyping: 2nd round multiplex PCR:

For 1 x:

2x buffer(with dNTPs and $Mgcl_2$)	12.5µl
Primer Con3 (20pmoles/ µl)	1 µl
Primer P4(20pmoles/ µl)	1 µl
Primer P6(20pmoles/ µl)	1 µl
Primer P8(20pmoles/ µl)	1 µl
Primer P9(20pmoles/ µl)	1 µl
Primer P10(20pmoles/ µl)	1 µl
Primer P11(20pmoles/ µl)	1 µl

Enzyme mix (Taq polymerase and Reverse transcriptase) 1µl

RNase free water 19.5 µl

1. 2nd round PCR mix was prepared.
2. 40 µl of PCR mix was added to each tube.
3. 10 µl of 1st round product was added. For negative control RNase free water10 µl was added
4. The tubes were transferred to the PCR machine room.
5. Brief spin was done in microcentrifuge
6. The tubes were added to the thermocycler
7.. For PCR, the following cycling conditions were set.

94 ºc	4 min	x1
94 ºc 45 ºc 72 ºc	1 min 2 min 1 min	x30
72 ºc 4 ºc	7 min hold	x1

ANALYSIS BY GEL ELECTROPHORESIS [48]

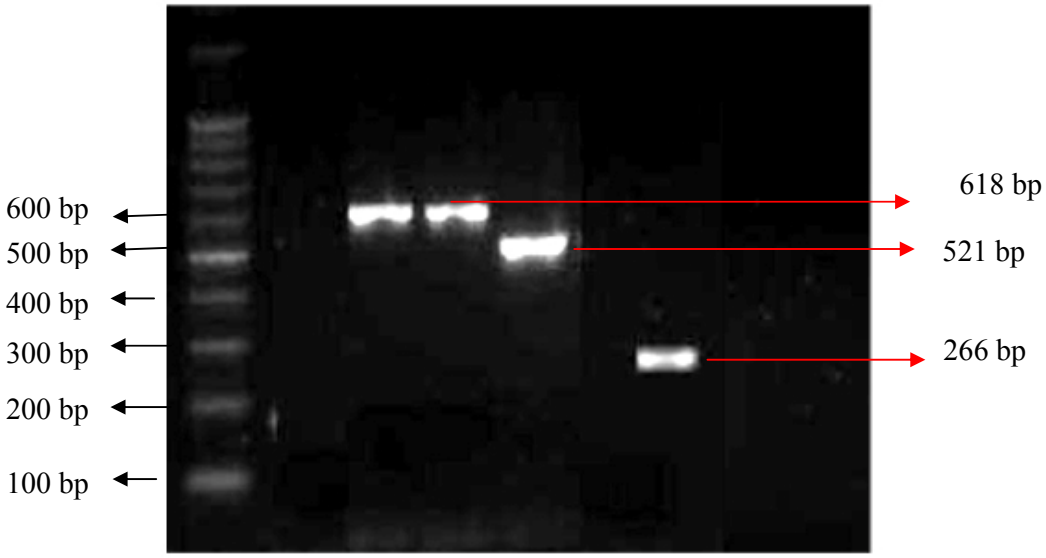
Preparation of agarose gel[89]: 1.To prepare 1% agarose gel 1.5gms agarose powder was mixed with 150ml of electrophoresis buffer, then heated in a microwave oven, mixed well until the agarose was uniformly dissolved. 2.After cooling to about

60ºC, ethidium bromide was added to the gel(final concentration 0.5 ug/ml) to facilitate visualization of DNA after electrophoresis. After cooling the solution, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature 3.After the gel hardens enough, the gel was mounted in electrophoresis tank. 4.Electrophoresis buffer is poured into the electrophoresis tank so that the gel was completely immersed. 5.The comb was carefully removed. 6.Ethidium Bromide is mutagenic and should be handled with extreme caution. Dispose of the contaminated tip into a dedicated ethidium bromide waste container.

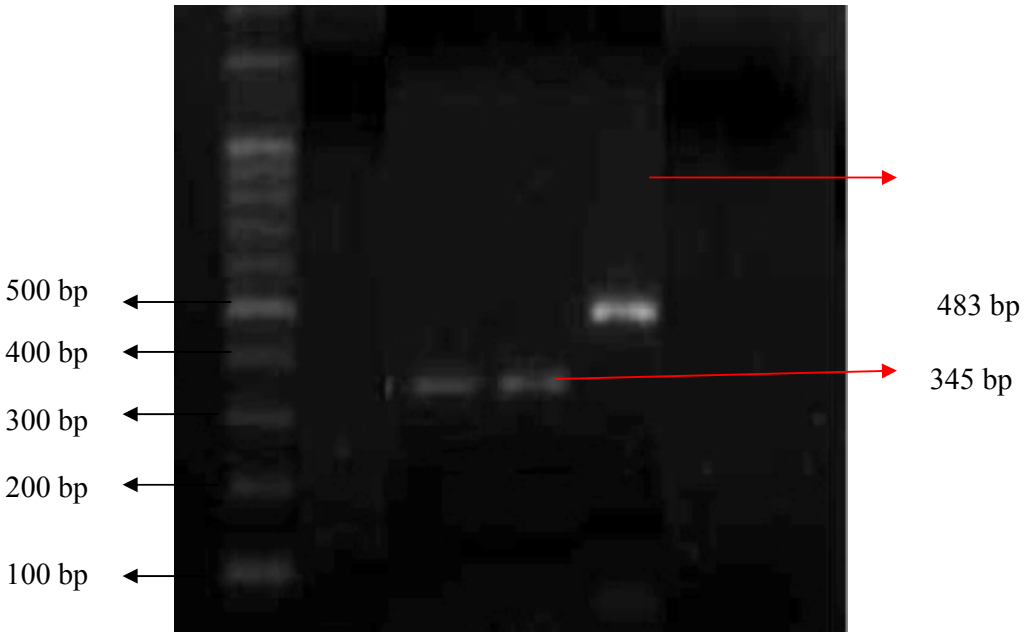
Gel electrophoresis:

1. Electrical leads were connected. As the DNA amplified by PCR was charged negative, it migrates from cathode to anode. 2. 2µ l of 6 x loading buffer was added to each tube containing the PCR reactant and mixed. The mixture was slowly loaded into the slots of the submerged gel using a Micropipette. Marker DNAs of known size was loaded into slots. Constant voltage of 50-150 V was applied to allow the gel run until the Bromophenol Blue have migrated ¾ the length of the gel. All PCR products were analysed in 1% agarose gel, stained with ethidium bromide and observed under UV transilluminator.

G genotyping



P genotyping



G Genotyping: First-amplification consensus primers:

VP7 F	5'ATG TAT GGT ATT GAA TAT ACC AC 3'	product size 881bp
VP7R	5' AAC TTG CCA CCA TTT TTT CC 3'	

Second amplification Type specific primers :

G1	5' CAA GTA CTC AAA TCA ATG ATG G 3'	product size 618bp
G2	5' CAA TGA TAT TAA CAC ATT TTC TGT G 3'	product size 521 bp
G3	5'ACG AAC TCA ACA CGA GAG G 3'	product size 682 bp
G4	5' CGT TTC TGG TGA GGA GTT G 3'	product size 452 bp
G5	5' GTC ACA CCA TTT GTA AAT TCG 3'	product size 754 bp
G9	5'CTA GAT GTA ACT ACA ACT AC 3'	product size 179 bp
G10	5'ATG TCA GAC TAC ARA TAC TGG 3'	product size 266 bp

P Genotyping:

First amplification consensus primers:

con3	5'TGG CTT CGC CAT TTT ATA GAC A3'	product size 876 bp
con2	5' ATT TCG GAC CAT TTA TAA CC 3'	

Second amplification Type specific primers:

P[8]	5'TCT ACT GGR TTR CAN TGC 3'	product size 345 bp
P[4]	5' CTA TTG TTA GAG GTT AGA GTC 3'	product size 483 bp
P[6]	5'TGT TGA TTA GTT GGA TTC AA 3'	product size 267 bp
P[9]	5'TGA GAC ATG CAA TTG GAC 3'	product size 391 bp
P[10]	5'ATC ATA GTT AGT AGT CGG 3'	product size 583 bp
P[11]	5' GTA AAC ATC CAG AAT GTG 3'	product size 312 bp

Observation And Results

OBSERVATION AND RESULTS

200 stool samples were collected from children less than 5 years of age which include study group of 150 children with diarrhoea and 50 controls without diarrhoea.

Bacterial culture was done for the samples and identified by standard microbiological procedures such as colony morphology and relevant bio-chemical reactions .

Saline wet mount , iodine wet mount and modified acid fast staining were done to identify parasites.

Rota virus antigen detection was done for all samples by ELISA. 24 samples showed positivity. Reverse Transcriptase Polymerase chain Reaction was done for determination of G and P genotype of the rota virus strains.

The results of these tests are as follows:

TABLE 1: Age distribution among study and control group

Age (months)	Study		Control	
	No.	**%**	**No.**	**%**
0 to 6	10	6.7	3.0	6.0
7 to 12	76	50.7	23	46
13 to 24	43	28.7	12	24
25 to 60	21	14	12	24
Total	150	100	50	100
Mean age	**16.2 ± 11 sd months**		**18.9±12.2 sd months**	
Difference of means	2.7			
't' value	1.456			
Degree of freedom	1.98			
p value	1.47			

Most of the children were in the age group of 7-12 months. The study and control group were similar in age distribution.

TABLE 2: Gender distribution of study (n=150) and control group (n=50)

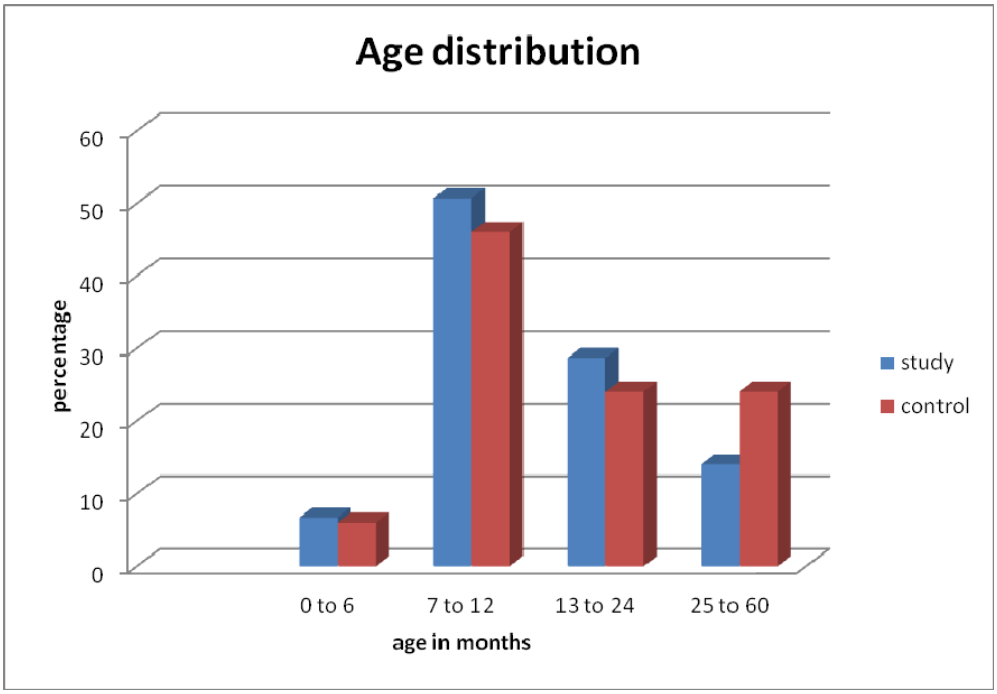
Age (mths)	Study group		Control group		Study group		Control group	
	Male	**%**	**male**	**%**	**female**	**%**	**female**	**%**
0 to 6	4	2.7	2	4	6	4	1	2
7 to 12	42	28	12	24	34	22.7	11	22
13 to 24	25	16.7	6	12	18	12	6	12
25 to 60	15	10	8	16	6	4	4	8
Total	**86**	**57.3**	**28**	**56**	**64**	**42.7**	**22**	**44**
Significance	p>0.05				p>0.05			

The study group and control group were similar in gender distribution. In the study group and control group, the male to female ratio was 1.3 :1.

TABLE 3: Duration of diarrhoea

Range	Mean duration
24 hrs to 120 hrs	60.6±30.9 hrs

The mean duration of diarrhoea in study group was 60.6±30.9 hrs.


Age distribution
60
50
40
30
20
10
0
percentage
0 to 6
7 to 12
13 to 24
25 to 60
age in months
study
control

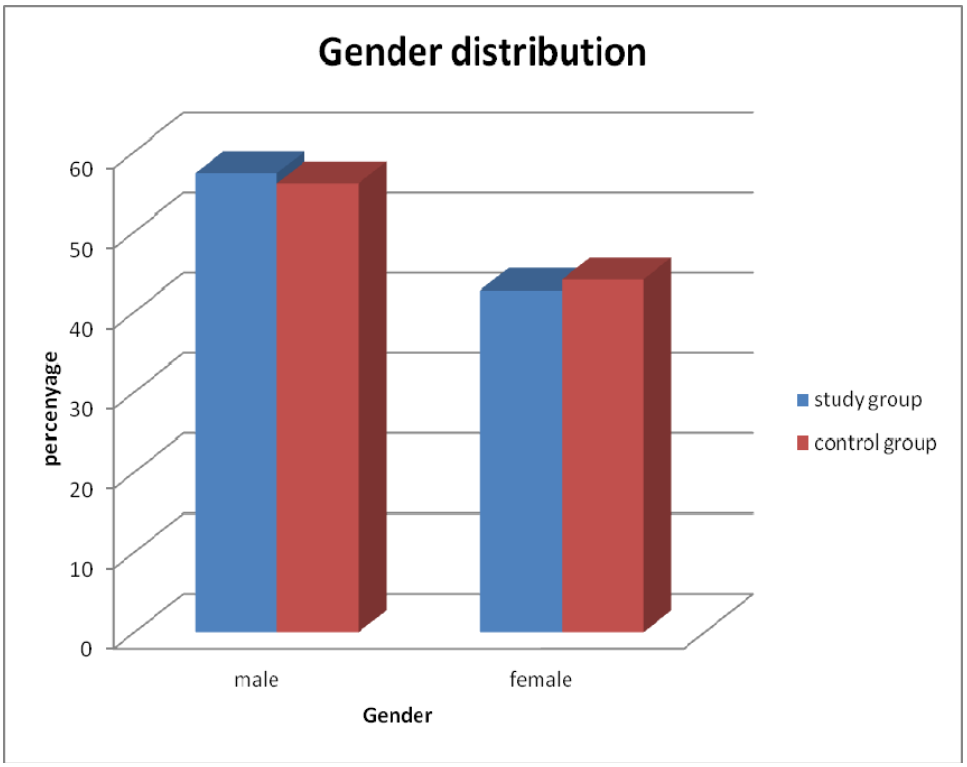

Gender distribution
60
50
40
30
20
10
0
percenyage
male
female
Gender
study group
control group

TABLE 4: Breast feeding status among study and control group

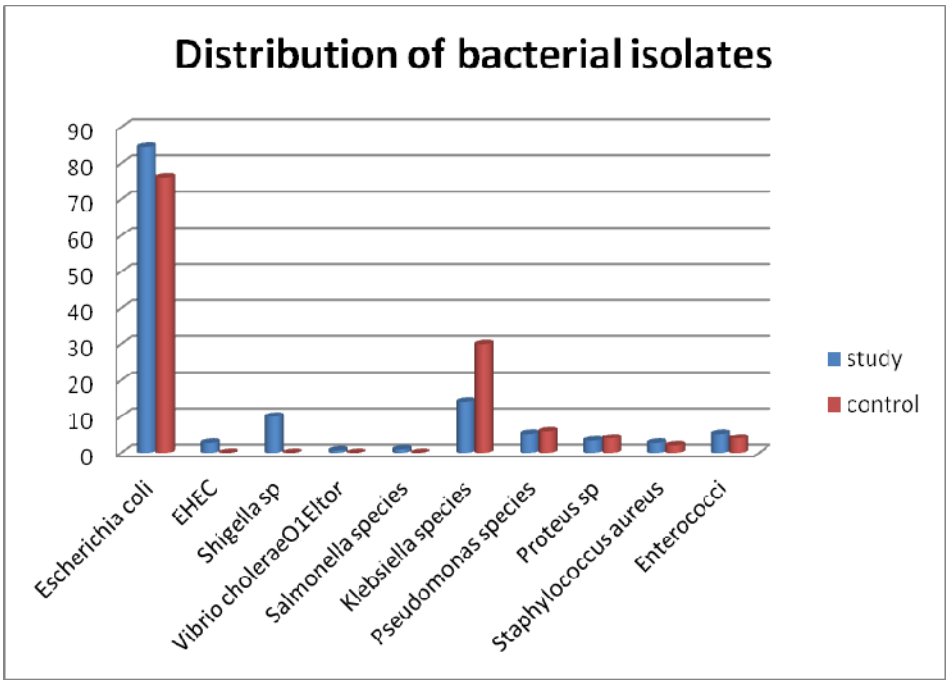
Breast feeding status	Study group		Control group		Total		Chisquare	degree of freedom	p value
	No.	%	No.	%	No.	%			
Children breast fed	83	55.3	39	78	122	61	8.099	1	<0.01
Children not breast fed	67	44.7	11	22	78	39			

The children in the control group had significant breast feeding status than the study group.

TABLE 5: Isolates identified from stool samples of study and control group

	Organism	Study group		Control group		Significance
		No.of org identified	%	No.of org identified	%	
Bacterial isolate	Escherichia coli	127	84.7	38	76	p>0.05
	EHEC(Enterohaemorrhagic E.coli)	4.0	2.7	0	0	-
	Shigella sp	15	10	0	0	-
	Vibrio choleraeO1Eltor	1	0.7	0	0	-
	Salmonella species	2	1	0	0	-
	Klebsiella species	21	14	15	30	p>0.05
	Pseudomonas species	8	5.3	3	6	p>0.05
	Proteus sp	5	3.3	2	4	p>0.05
	Staphylococcus aureus	4	2.7	1	2	p>0.05
	Enterococci	8	5.3	2	4	p>0.05
Parasites	Giardia lamblia	2	1.3	0	0	-
	Cryptosporidium parvum	2	1.3	0	0	-
	Entamoeba histolytica cyst	5	3.3	1	2	P>0.05
	Ascaris lumbricoides	1	0.7	0	0	-
Virus	Rota virus	24	16	0	0	-

Rota virus (16%)were identified significantly in the study group followed by Shigella isolates(10%), EHEC isolates(2.7%), Salmonella isolates (1 %), Vibrio cholerae isolates(0.7%) .


Distribution of bacterial isolates
90
80
70
60
50
40
30
20
10
0
Escherichia coli
EHEC
Shigella sp
Vibrio choleraeO1Eltor
Salmonella species
Klebsiella species
Pseudomonas species
Proteus sp
Staphylococcus aureus
Enterococci
study
control

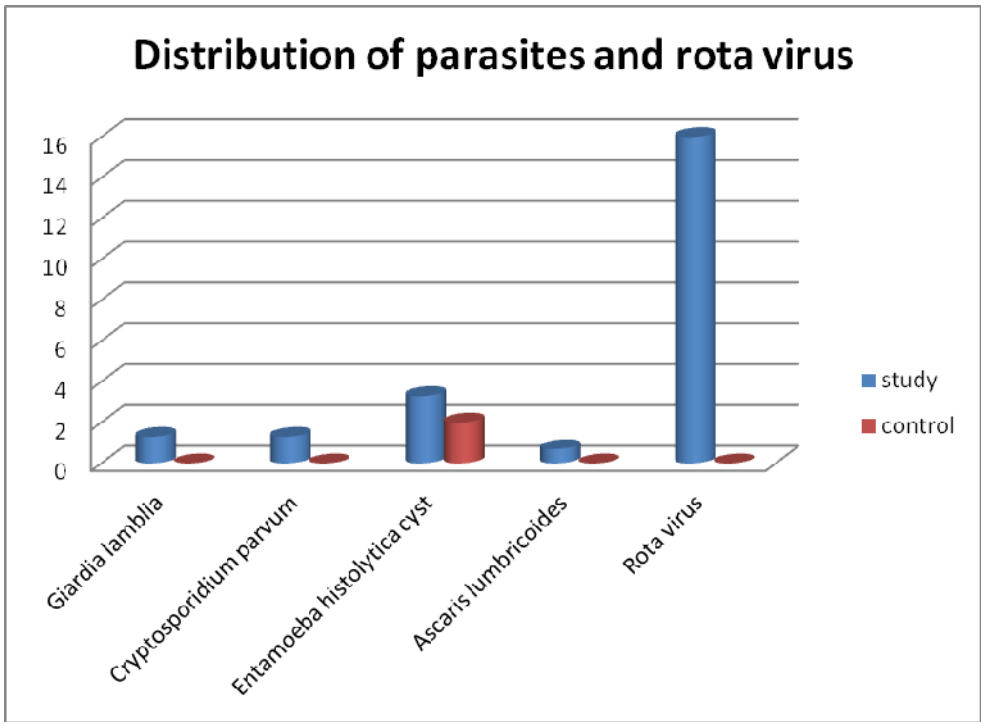
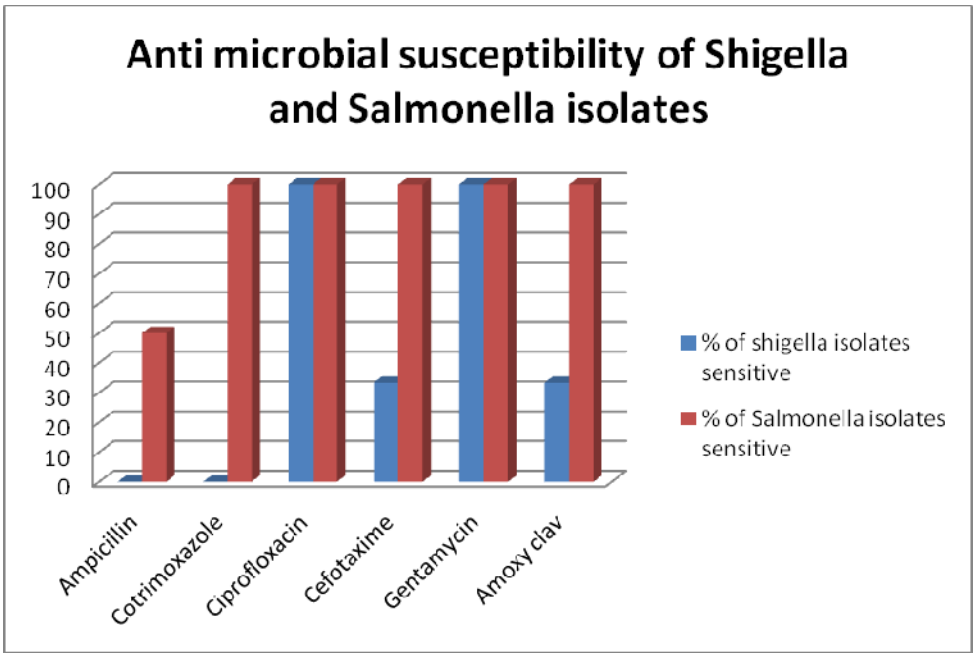

Distribution of parasites and rota virus
16
14
12
10
8
6
4
2
0
Giardia lamblia
Cryptosporidium parvum
Entamoeba histolytica cyst
Ascaris lumbricoides
Rota virus
study
control

TABLE 6: Antimicrobial susceptibility of isolates of Shigella(n=15), Salmonella (n=2) and Vibrio cholerae (n=1) isolates

	No. of Shigella isolates sensitive	%	No.of Salmonella isolates sensitive	%	No.of Vibrio isolates sensitive	%
Ampicillin 10 mcg	0	0	1	50	1	100
Cotrimoxazole 1.25/3.75 mcg	0	0	2	100	0	0
Ciprofloxacin 5 mcg	15	100	2	100	-	-
Cefotaxime 30 mcg	9	33.3	2	100	-	-
Gentamycin 10 mcg	15	100	2	100	-	-
Amoxy clav 20/10 mcg	9	33.3	2	100	-	-
Chloramphenicol 30 mcg	-	-	-	-	1	100
Tetracycline 30 mcg	-	-	-	-	1	100

All the Shigella isolates were sensitive to ciprofloxacin, gentamycin and none of them sensitive to ampicillin and cotrimoxazole. All the Salmonella isolates were sensitive to ciprofloxacin, gentamycin,cefotaxime , amoxy clavulanic acid and cotrimoxazole. Vibrio cholerae was sensitive to Ampicillin, Chloramphenicol, tetracycline and resistant to cotrimoxazole.


Anti microbial susceptibility of Shigella and Salmonella isolates
100
90
80
70
60
50
40
30
20
10
0
Ampicillin
Cotrimoxazole
Ciprofloxacin
Cefotaxime
Gentamycin
Amoxy clav
% of shigella isolates sensitive
% of Salmonella isolates sensitive

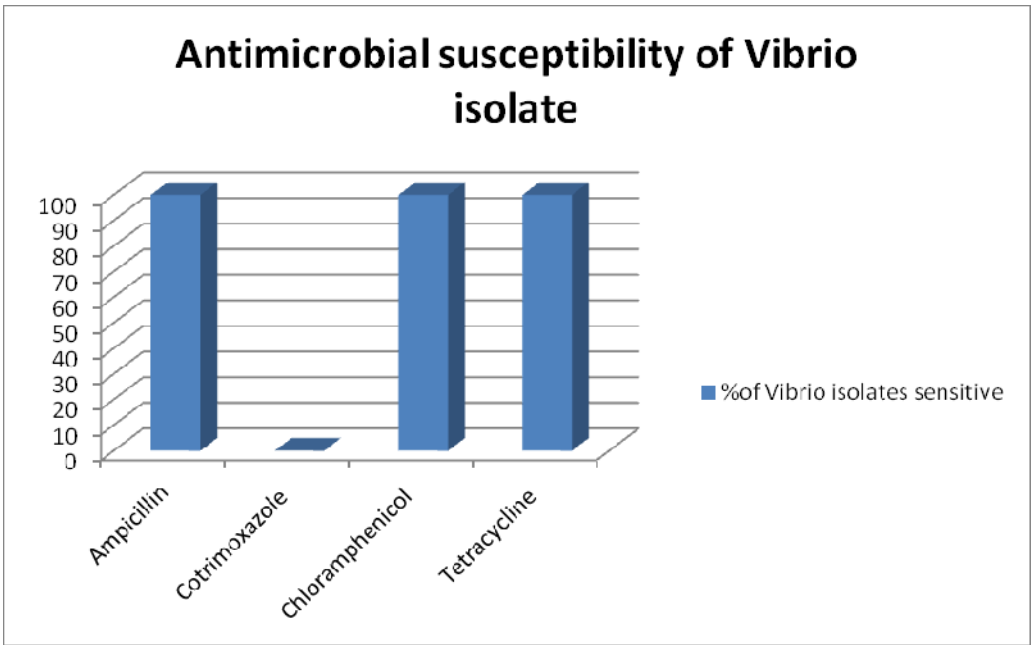

Antimicrobial susceptibility of Vibrio isolate
100
90
80
70
60
50
40
30
20
10
0
Ampicillin
Cotrimoxazole
Chloramphenicol
Tetracycline
%of Vibrio isolates sensitive

TABLE 7: Breast feeding status among rota virus positive patients and non rota virus patients

Breast feeding status	Rotavirus patients		Non rotavirus patients		p value	deg of freedom	Chisquare
	No.	%	No.	%			
children breast fed	11	45.8	72	57.1	>0.05	1	1.043
children not breast fed	13	54.2	54	42.9			

Breast feeding status was found in 45.8% of rota virus patients and 57.1% of non rotavirus patients.

TABLE 8: Selected clinical data of the most important etiological agents

Organism	No. of patients	No. of patients with				
		watery diarrhoea	bloody diarrhoea	Fever	abdominal pain	vomiting
Rota virus	24	24	0	16	0	20
Shigella spp	15	10	10	11	09	05
Salmonella spp	2	2	1	2	2	1
Vibrio cholerae	1	1	0	0	0	1
Giardia lamblia	2	2	0	0	2	0
Cryptosporidium parvum	2	2	0	0	2	0

Rota virus and Vibrio cholerae infected patients presented as watery diarrhoea associated with vomiting. Shigella and Salmonella infected patients presented as bloody diarrhoea associated with abdominal pain and fever. Cryptosporidium and Giardia infected patients presented as watery diarrhoea and abdominal pain.

TABLE 9:

Correlation of clinical severity in rota virus and non rota virus patients

Clinical symptom	Rotavirus diarrhoea patients(n=24)		Non rota diarrhoea patients(n=126)		p value
	No.	%	No.	%	
Fever	16	66.7	77	61.1	p>0.05
≥6 watery stools per day	17	70.8	50	39.7	p<0.01
Vomiting	20	83.3	67	53.2	p<0.001
vomiting ≥4 times per 24 hrs	11	45.8	27	21.4	p<0.05
bloody stools	0	0	20	15.9	-

A significantly higher proportion of rota virus patients than non rota virus patients were associated with more than 6 watery stools per day(70.8%) and increased bouts of vomiting (45.8%).

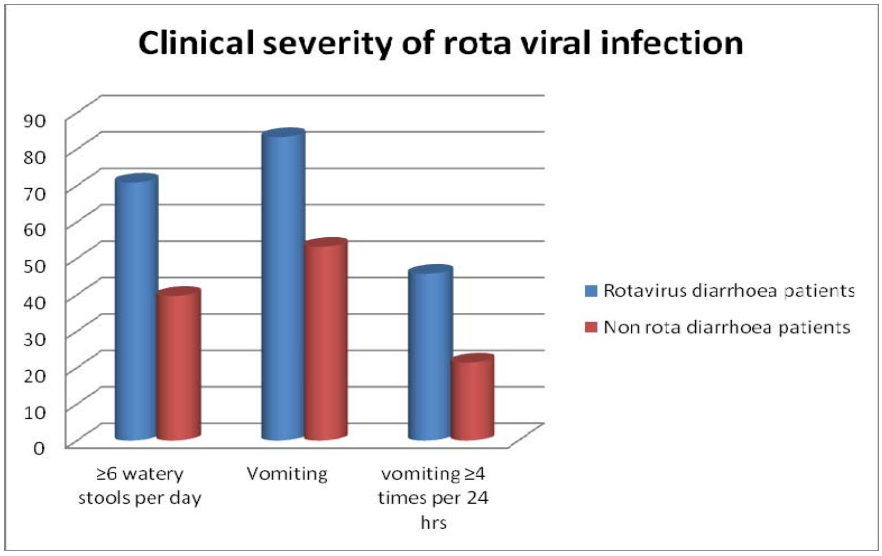


TABLE 10: Distribution of severe dehydration by age group among rota virus and non rota virus diarrhoea patients

Age group (mths)	Rotavirus diarrhoea(n=24)	%	Non rotavirus diarrhoea(n=126)	%	p value
0-6	0	0	1	0.8	-
7-12	4	16.7	12	9.5	p>0.05
13-24	3	12.5	3	2.4	p>0.05
25-60	0	0	2	1.6	-
Total	7	29.2	18	14.3	p>0.05

29.2% of rota virus patients were associated with severe dehydration out of which most of them were in 7-12 months age group.

TABLE 11: Age wise analysis of group A rota virus infection among children 0-5 years with diarrhoea

Age in mths	Rotavirus patients(n=24)		Non rotavirus patients(n=126)		p value
	No.	%	No.	%	
0-6	2	8.3	8	6.3	p>0.05
7-12	17	70.8	59	46.8	p<0.05
13-24	4	16.7	37	29.4	p>0.05
25-60	1	4.2	22	17.5	p<0.05

Overall rota virus infection rate of 16% was found among children with acute diarrhoea. Rota virus positive children were significantly higher in the age group 7-12 months and significantly lesser in the age group 25-60 months.

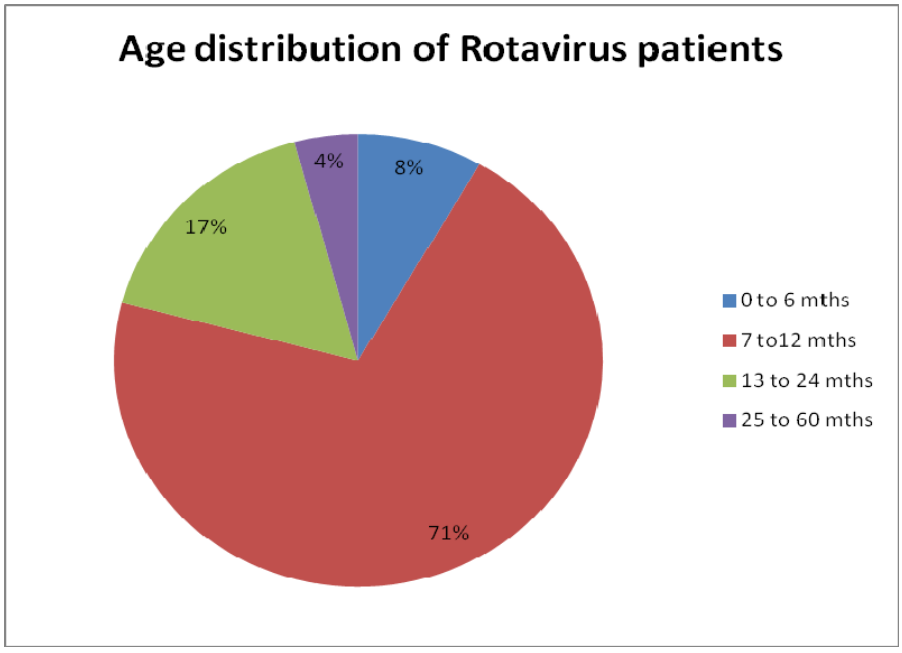


TABLE 12: Gender wise analysis of rota virus patients and non rota virus patients

	male(n=86)		female(64)		p value
	No.	**%**	**No.**	**%**	
Rotavirus patients(n=24)	15	17.4	9.0	14.1	p>0.05
Non rotavirus patients(n=126)	71	82.6	55	85.9	

The incidence of rota virus was 17.4% in male children and 14.1% in female children.

TABLE 13: Group A rota virus types detected by RT-PCR(n=12)

No of strains typed by RT-PCR	12	100%
No of strains fully typed	8	66.7%
No of strains partially typed	1	8.3%
No of strains untypeable	3	25%

Out of 24 rota virus strains ,12 were subjected to typing by RT PCR. Out of 12 strains ,66.67% were fully typed for G and P genotype,8.33% were partially typed and 25% were not typeable.

TABLE 14: Distribution of typed and untyped rota virus strains

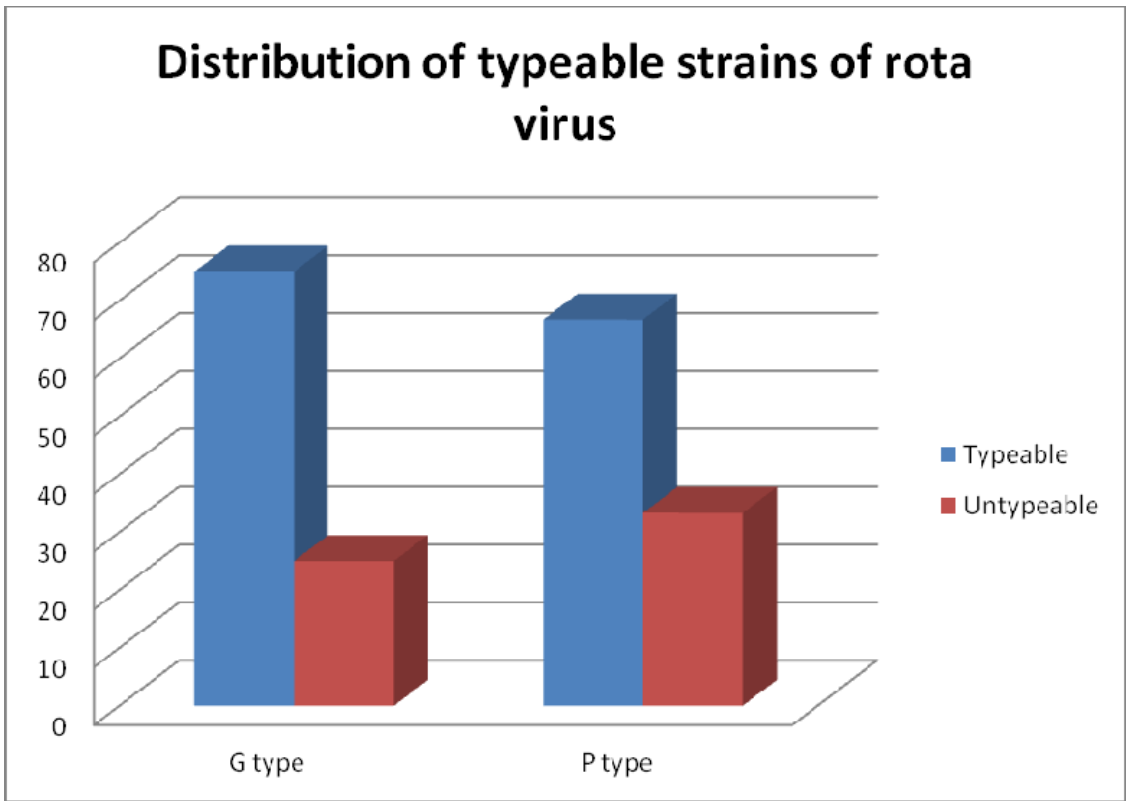
	G type	P type	Total strains
Typeable	9(75%)	8(66.7)	12(100%)
Nontypeable	3(25%)	4(33.3)	12(100%)

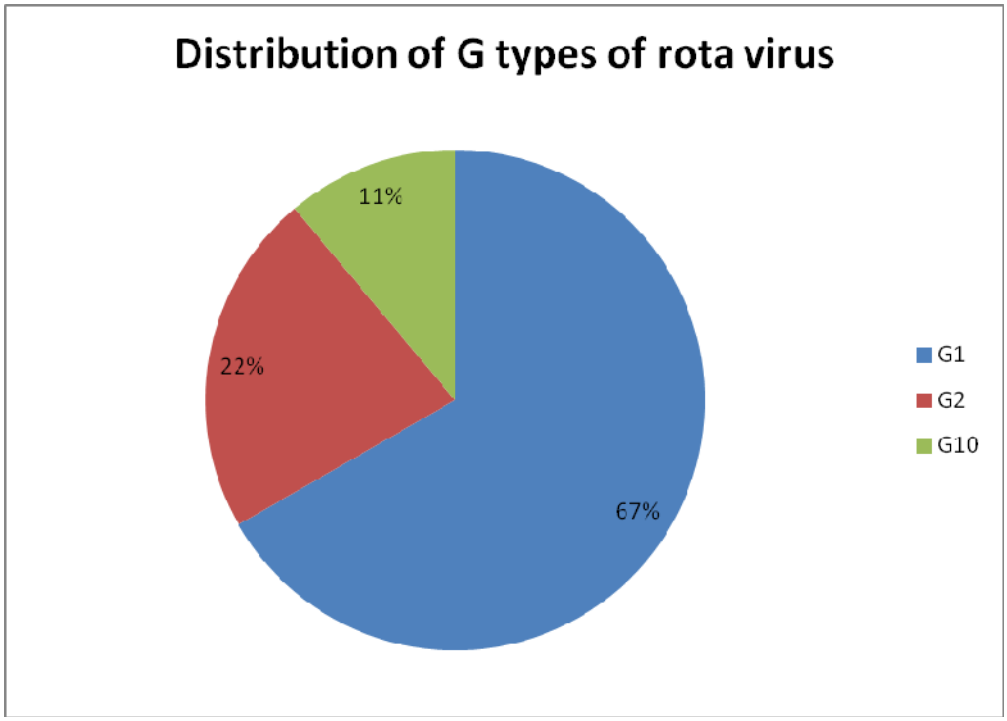
Out of 12 rota virus strains, 75% of G genotype and 66.7% of P genotype could be determined. 25% of G genotype and 33.3% of P genotype could not be determined.

TABLE 15: Distribution of G and P genotype of group A rota virus detected among under five children with diarrhoea

	Genotype	No of strains	%
G type(n=9)	G1	6	66.7
	G2	2	22.2
	G10	1	11.1
P type(n=8)	P[8]	6	75
	P[4]	2	25

The G1 genotype was identified in 66.7% of rota virus strains, followed by G2 in 22.2% and G10 in 11.1%. P[8] was identified in 75% of the strains and P[4] in 25% of the strains.


Distribution of typeable strains of rota virus
80
70
60
50
40
30
20
10
0
G type
P type
Typeable
Untypeable


Distribution of G types of rota virus
11%
22%
67%
G1
G2
G10

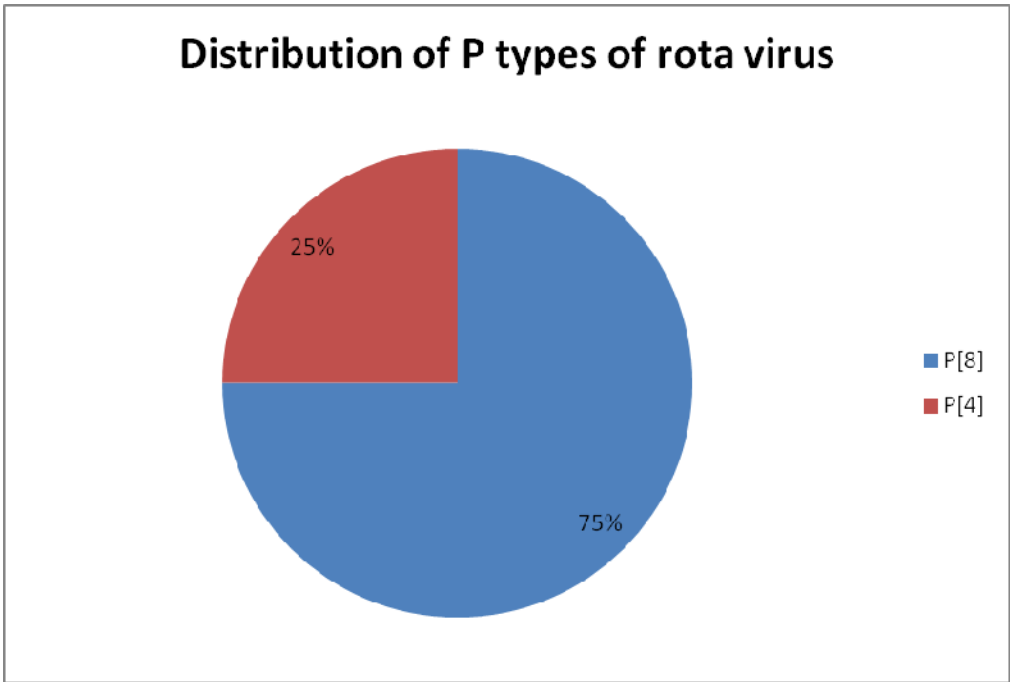
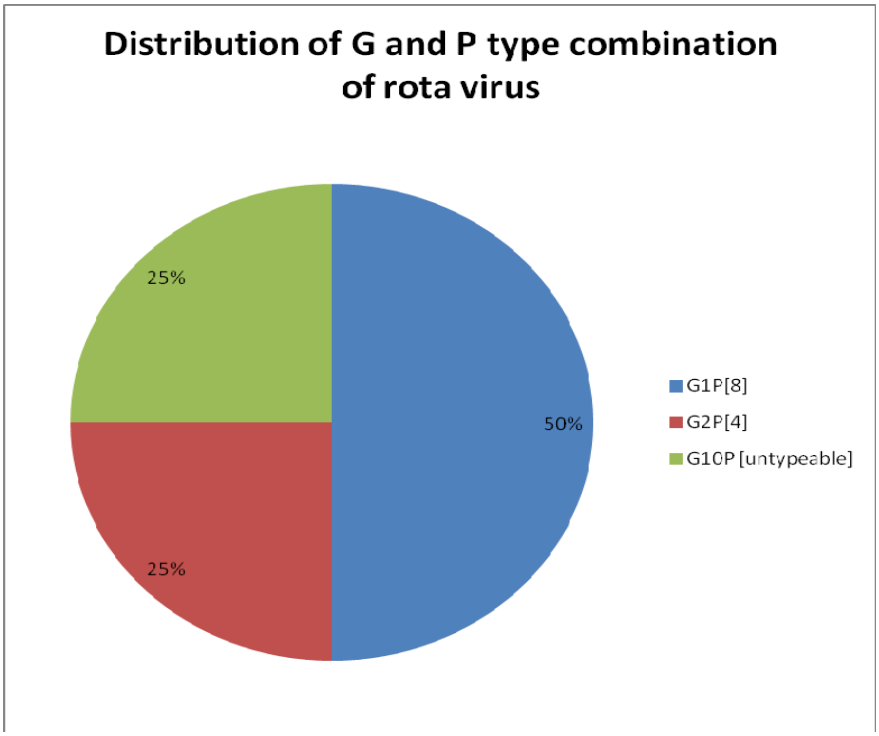

Distribution of P types of rota virus
25%
75%
P[8]
P[4]

TABLE 16: Distribution of G and P type combination of group A rota virus strains detected among under five children with diarrhoea

GP type	No of strains	%
G1P[8]	6	50
G2P[4]	2	25
G10P [untypeable]	1	25
Total	9	100

G1P[8] was determined in 50% of the strains, followed by G2P[4] in 25% and G10P[untypeable] in 25% of the strains.



Discussion

DISCUSSION

Acute infectious diarrhoea is one of the major causes of mortality in children, particularly in developing countries like India. Children under 3 years of age may experience as many as 10 episodes of diarrhoea per year[47]. Rotavirus is among the most common causes of diarrhoea worldwide[18,47] accounting for 134 million episodes/year[47,92,93].

Effective control of rota viral disease in any community depends upon an accurate understanding of disease burden and knowledge of the relative importance of circulating serotypes. This study was conducted to determine bacterial, parasitic profile and rotavirus detection and characterization of rota virus in children under 5 years of age with acute diarrhoea.

In the present study, the study and control group were similar in age distribution. Most of the children in the study group(50.7%) were in the age group of 7-12 months..(Table 1). This correlates with John Albert, M. et al[39] study in which 58.5% of diarrhoeal patients were in the age group 0-12 months.

In the present study, the study group and control group were similar in gender distribution. In the study group and control group, the male to female ratio was 1.3:1. (Table 2) Thus both the study and control groups were comparable. The present study is in accordance with the study conducted by Mohammad Youssef et al[60] study in which male and female ratio was 1.3:1 and in John Albert, M. et al[39] study ,the ratio was 1:0.7.

In the study group, the mean duration of diarrhoea was 60.6±30.9 hrs. (Table 3). This study correlates with study by Nakawesi et al[64] in which the mean duration of diarrhoea was 90 hrs.

In this study 55.3% and 78% of children were breast fed in study and control group respectively. (Table 4). Since the breast feeding rate was low in study group, they were more prone to infection and diarrhoea.

In the present study, Rota virus was detected in 16% of the study group. The true incidence of rota virus could be higher than 16% as this data represents only children with diarrhoea requiring medical attention.

Distribution of rota virus in different studies:

Study	**%**
Present study	16
P.Saravanan et al[72]	22.5
S. S. R. Ajjampur et al[91]	25.3
Gagandeep Kang et al[30] study	33

Rota virus was not detected from any one of the control group. This implies that rota virus was significantly associated with diarrhoea.(Table 7). This correlates with the study of M.Aminu et al[55] in which rota virus was not detected in controls.

Distribution of bacterial pathogens in different studies

Study	**Organism**	**%**
Present study	Shigella spp	10
John Albert et al[39]	Shigella spp	9.2
Gopinath Balakrish Nair et al[32]	Shigella spp	7.9
Present study	Vibrio cholerae	0.7
S. S. R. Ajjampur et al[91]	Vibrio cholerae	0.6
Present study	Salmonella spp	1
Iruka N et al[36]	Salmonella spp	0.9
Present study	EHEC	2.7
Patrick et al[77a]	EHEC	1.4

The detection of EHEC would avoid unnecessary diagnostic imagings and surgeries on patients infected with EHEC in search of cause of bloody diarrhoea. Also it avoids deleterious antimicrobial and antimotility treatment. Because quinolones which are prescribed for majority of patients are potent inducers of shiga toxin encoding bacteriophages and toxin production in EHEC, early detection helps in identification and monitoring of patients at risk of progression to haemolytic uraemic syndrome.

The above mentioned pathogens were obtained only from study group and none of these isolates from control group. The bacterial pathogens Shigella spp and Salmonella spp were confined mainly to the study group. This correlates with the

study by T.I.Ogunsanya et al[95]in which Shigella spp and Salmonella spp.were confined mainly to the diarrhoeal group.

Distribution of parasites in different studies:

Study	Organism	%
Present study	Giardia lamblia	1.3
S. S. R. Ajjampur et al[91]	Giardia lamblia	0.63
Present study	Cryptosporidium parvum	1.3
S Das et al[90]	Cryptosporidium parvum	1.4
Mohammad Youssef et al [60]	Cryptosporidium parvum	1.5
Present study	Entamoeba histolytica	3.3
S. Das et al[90]	Entamoeba histolytica	4.8

Giardia lamblia and Cryptosporidium were not identified in controls thus significantly associated with diarrhoea. This correlates with the study by S. S. R. Ajjampur et al[91] in which Giardia and Cryptosporidium were not identified in controls.

Antimicrobial sensitivity of significant isolates in different studies:

Study	Isolate	Sensitivity pattern
Present study	Shigella	100% sensitive to ciprofloxacin and gentamycin and 0% sensitive to ampicillin and cotrimoxazole

Roger L.Shapiro et al[85]	Shigella	100% sensitive to ciprofloxacin and gentamycin and 0% sensitive to ampicillin and cotrimoxazole
S.Das et al[90]	Shigella	100% sensitive to ciprofloxacin and gentamycin and 0% sensitive to cotrimoxazole
Present study	Salmonella	100% were sensitive to ciprofloxacin, gentamycin,cefotaxime, cotrimoxazole ,amoxy clavulanic acid
Moyo et al[62]	Salmonella	all the isolates(100%) were sensitive to ciprofloxacin, cefotaxime
Present study	Vibrio cholerae	sensitive to ampicillin, chloramphenicol, , tetracycline and resistant to cotrimoxazole
Roger L.Shapiro et al[85]	Vibrio cholerae	98% sensitive to tetracycline, 93% to ampicillin 100% resistant to cotrimoxazole.

In the present study, most of the bacterial pathogens showed high rate of resistance to ampicillin and cotrimoxazole which are used for empirical treatment and are readily available over the counter. The high rate of resistance to these antibiotics may be because of their indiscriminate use. Hence periodic monitoring of drug susceptibility and local information of drug resistance is mandatory for providing treatment guidelines for diarrhoea.

In the present study, breast feeding status was found in 45.8% of rota virus patients and did not demonstrate the protective effect of breast feeding against rotavirus diarrhoea.(Table 7). This is in accordance with the study by Wobudeya et

al[108] and Nakawesi et al[64] and Linhares et al[52] in which they have found no evidence of protection of breast feeding against rotavirus diarrhoea .

Since most of the children in the study were above six months of age and on complimentary feeding, the contribution of breast feeding could not be determined

According to Ebina[24a] et al, the intestinal mucosa needs continuous bathing with antibodies and other anti infective components in breast milk for protection against rota virus. So breast feeding may be protective only if it is practised with intensity and frequency that allows continuous high protection of the mucosa rather than the sporadic small volumes.

In our study, Shigella infected patients presented as bloody diarrhoea(66.7%)and fever(73.3%).(Table 8).This is in accordance with the study by Mohammad Youssef et al[60] in which 60% of Shigella infected patients presented as bloody diarrhoea and fever.

Rota virus infected patients presented as watery diarrhoea associated with vomiting. A significantly higher proportion of rota virus patients than non rota virus patients were associated with more than 6 watery stools per day(70.8%) and increased bouts of vomiting (45.8%). (Table 9). This study correlates with the study of Khitam Muhsen et al[46] in which rota virus infected children significantly presented with more watery stools (87.1%) and more vomiting(57.6%). This shows that rota virus is associated with more severe illness than other enteropathogens.

In the present study, 29.2% of rota virus patients were associated with severe dehydration out of which most of them were in 7-12 months age group.(Table 10). This correlates with the study by A.K. Siddique et al[1] in 16% of rotavirus patients presented with severe dehydration and most of them were in 6-11 months age group. This shows that children in 7-12 months of age group are at higher risk of death due to severe dehydration associated with rota virus infection.

In the present study, rota virus positive children were significantly higher in the age group 7-12 months and significantly lesser in the age group 25-60 months.(Table 11). The present study correlates with the study of P.Saravanan et al[72] , M.John Albert et al[39] study and A.K.Siddique et al[1] in which majority of rota virus positive patients were in the age group 7-12 months.

This could be because of protective effect of maternal antibodies in infants<6 months of age and by the development of natural immunity after successive infections in children >2 years of age. This also indicates that prophylactic measures such as vaccination should be taken in the first few months of life to prevent rota viral infection.

The incidence of rota virus was 17.4% in male children and 14.1% in female children and the incidence rate was similar in both male and female children.(Table 12) This correlates with the study of P.Saravanan et al[72] in India in which no association was found between male(23.9%) and female(21.1%). Another study by R.N kalaf and K.S Ghenghesh[80] showed similar rates among male and female patients,33.6%(40/119) and 32.1%(26/81). Male and female ratio among rota virus

positive patients was 1.2:1 in the present study. This correlates with Alicia Sa´nchez-Fauquier et al[5] study which showed male:female ratio of 1.3:1.

In the present study, 66.7% were fully typed for G and P genotype,8.3% were partially typed and 25% were not typeable(Table 13). This was similar to Vivek jain et al[103] study which has showed 12% of partially or nontypeable strains in India. Moyo et al study[61b] showed 6.1% of nontypeable strains and 6% of partially typeable strains in Tanzania.

In the present study , 75% of G genotype and 66.67% of P genotype could be determined. 25% of G genotype and 33.33% of P genotype could not be determined.(Table 14) . This correlates with the review study of N.A.Cunliffe et al[63] in which 26% of G genotype and 32% of P genotype could not be typeable. In a review study by Ali M.Kheyami et al[4] , 11-31.3% of rota virus strains could not be typeable for G type. In a study by Zuccotti et al[109] ,19.8 % of rota virus strains could not be typeable for P type.

The nontypeability of strains may be due to the emergence of new strains. Since rotaviruses genetically mutate, it is to be expected that sometimes RT-PCR methodologies are unable to identify all types. Another reason is that the strain untypeable could be unrecognised which needs further investigation as stated by M.Aminu et al[55].

In the present study, G1 genotype was identified in 66.7% of rota virus strains being the most predominant type followed by G2 in 22.2% and G10 in 11.1%. (Table

15). This correlates with the study of Malek MA et al[56] in which G1 was the predominant type (80%). Ryuichi Uchida et al[88] study showed predominance of G1 type (77%) among children with rotavirus diarrhoea. Also studies done by Alicia Sa´nchez-Fauquier et al[5], Zuccotti et al[109] ,NishioO et al[68], Aminu.M et al[55] , Ali M.Kheyami et al[4], N.A.Cunliffe et al[63] showed predominance of G1 type in rota virus strains.

Other than the globally common genotypes G1 and G2, G10 genotype was also seen in the present study. G10 genotype has been reported in the study by Gagandeep Kang et al[30] in children with diarrhoea in south India. But previous studies by Dunn et al[23] and Sukumaran M et al[94] have reported G10 type in asymptomatic newborn infants. This implies that G10 genotype is increasingly being associated with symptomatic diarrhoea. G10 has also been reported in the study by Zuccotti et al[109].

In the present study, P[8] was the predominant P type (75%) followed by P[4] 25%. (Table 15) .This correlates with the study by Buesa J et al[14](77.1% of P[8]) and Zuccotti et al[109] in which only p[8] and p[4] types were seen with predominance of p[8]. The studies by Ryuichi Uchida et al[88] , Malek MA et al[56] and Moyo et al[61b] showed predominance of P[8] type of about 80-100%.

In the present study, G1P[8] was determined as the most common combination of G and P type(50%), followed by G2P[4] and G10P[untypeable].(Table16). G1P[8] andG2P[4] have been described as globally common genotypes in a review study done by Gentsch et al[31]. This study correlates with Khitam Muhsen et al[46] study which has

shown 49.1% of G1P[8] combination and Buesa J et al[14] study in which 42.7% of G1P[8] combination was found and Foster et al[29] study has shown 40.3% of G1P[8] combination. Ryuichi Uchida et al[88] study have also reported predominance of G1P[8] combination(70%).

Summary

SUMMARY

Stool samples were collected from 150 children with diarrhoea and 50 children without diarrhoea. The samples were subjected to saline wet mount, iodine mount,modified acid fast staining and culture.The isolates were identified by gram staining and biochemical reactions. Rota virus antigen was detected in the samples by VP6 specific monoclonal antibodies in Antigen capture Enzyme Linked immuno Sorbent Assay . The samples which were positive for rota virus were subjected to Reverse Transcriptase Polymerase Chain Reaction for characterization of the rota virus strains.

In the present study , both the study and control groups were comparable. Diarrhoea was common in the age group of 7-12 months of age followed by13-24 months.

The children in the study group had breast feeding status significantly lesser than the control group.

Among the bacterial isolates, Shigella sp, Vibrio sp, Salmonella sp and EHEC were seen significantly in the study group children with diarrhoea. All of the Shigella and Salmonella isolates were sensitive to ciprofloxacin ,gentamycin and none of the isolates were sensitive to ampicillin , cotrimoxazole .

Vibrio isolates were sensitive to ampicillin, chloramphenicol, tetracycline and resistant to cotrimoxazole.

Among the parasites,Giardia lamblia and Cryptosporidium oocysts were significantly observed only in the study group.

Rota virus incidence was found to be 16% and it was significantly associated with diarrhoea.

This study could not demonstrate the protective effect of breast feeding against rotavirus diarrhoea as most of the children were above six months of age.

The main clinical presentation of rota virus infected patients was watery diarrhoea associated with vomiting and fever.

When compared with non rotavirus patients, rota virus patients presented significantly with more than 6 watery stools, with frequent episodes of vomiting .

Rota virus patients were associated with severe dehydration out of which most of them were in 7-12 months age group.

Major proportion of the rota virus positive cases fell significantly in the age group between 7-12 months and no significant association was found in gender distribution.

66.7% of the rotavirus strains were fully typed for G and P genotype,8.3% were partially typed and 25% were not typeable.

75% of G genotype and 66.7% of P genotype could be determined.

G1 genotype was identified as the most predominant type followed by G2.

Along with the globally common genotypes G1 and G2, G10 genotype was also seen.

P[8] was the predominant P type identified followed by P[4] .

G1P[8] was determined as the most common combination of G and P type followed by G2P[4] and G10P[untypeable].

Conclusion

CONCLUSION

In the present study, rota virus, Shigella sp, Vibrio sp, Salmonella sp, EHEC, Giardia lamblia and Cryptosporidium were seen significantly in children with diarrhoea. All of the Shigella and Salmonella isolates were sensitive to ciprofloxacin and gentamycin. Vibrio cholerae was sensitive to tetracycline which is used for chemoprophylaxis and thus holds good still now. High rate of resistance of bacterial isolates to cotrimoxazole and ampicillin was seen which recommends continuous antibiotic surveillance to monitor development of resistance.

Rota virus incidence was found to be 16% among children under five years of age with diarrhoea. Majority of them were in the age group of 7 to 12 months associated with severe dehydration and protective effect of breast feeding was not found against rota virus infection in this age group. The genotype combination of rota virus identified were G1P[8],G2P[4] and G10P[untypeable]. Thus the present study shows significant association of rota virus in children under five years of age with acute diarrhoea and the prevalence of uncommon genotype G10.

The significant association of rota virus in childhood diarrhoea along with clinical severity suggests consideration of a rota virus vaccine in the childhood immunization program. In developing countries like India, exposure to an environment contaminated with human and animal faeces and close contact with animals in the domestic environment are factors enabling viral reassortment and the emergence of new strains.

Most of the rota virus strains in circulation appear to be like those of common genotypes G1-4 which are components of current vaccine. But the non typeable strains pose an unknown antigenic challenge to the current vaccine Rotateq. Hence continuous and prospective monitoring of circulating strains in a large scale study will detect any change in the distribution of rotavirus strains which would influence the vaccine based preventive strategies in India.

Appendix

APPENDIX I

1. Saline(0.9%Nacl):

Nacl	0.9g
Distilled water	100ml

Dissolve the Nacl in distilled water.sterilize by autoclaving.

2. Iodine solution;

Lugol's iodine diluted 5 times with distilled water.

Lugol's iodine:

Iodine crystals	5g
Potassium iodide	10g
Distilled water	100ml

Potassium iodide is dissolved in distilled water and iodine crystals are added slowly.The solution is filtered and kept in stoppered bottle of amber colour.

3. Kinyoun's Modified Acid Fast Staining:

Reagents:

1. 50% ethanol

   Add 50 ml of absolute ethanol to 50 ml distilled water.

2. Kinyoun's carbol fuchsin

   Dissolve 4 g of basic fuchsin in 20 ml of 95% ethanol(solution A)

   Dissolve 8 g of phenol crystals in 100 ml of distilled water.(solutionB)

   Mix solution A and B.

3. 1% sulphuric acid

   Add 1 ml of conc.sulphuric acid to 99 ml of distilled water.

4. Loeffler's alkaline methylene blue

   Dissolve 0.3 g of methylene blue in 30 ml of 95% ethanol.

   Add 100 ml of dilute(0.01%)potassium hydroxide.

APPENDIX II

1.Mac Conkey agar

Peptone	20g
Sodium taurocholate	5g
Agar	20g
Neutral red solution,	2% in 50% ethanol 3.5g
Lactose,	10 % aqueous solution 100ml

Dissolve the peptone and taurocholate in the water by heating. Add the agar and dissolve it in the steamer or autoclave. If necessary, clear by filtration. Adjust the pH 7.5.

2.Xylose lysine deoxycholate agar

Yeast extract	3g
Xylose	3.75g
Lactose	7.5g
Sucrose	7.5g
L-Lysine	5.0g
Sodium Chloride	5.0g
Sodium deoxycholate	2.5g
Sodium thiosulphate	6.8g
Ferric ammonium citrate	0.8g
Phenol red	0.08g
Agar	15 g
Water	1 Litre

Except for the deoxycholate, thiosulphate and ferric ammonium citrate, dissolve the ingredients in the water by autoclaving. Cool to 50˚c and add 20ml of a solution of sodium thiosulphate 34% and ferric ammonium citrate 4%, and 25 ml of a solution of sodium deoxycholate 10 %. Adjust the pH to 7.4.

3.Thiosulphate citrate bilesalt sucrose Agar:

Composition	grams/litre
Proteose peptone	10.0
Yeast extract	5
Sodium thiosulphate	10
Sodium citrate	10
Oxgall	8
Sucrose	20
Sodium chloride	10
Ferric citrate	1
Bromothymol blue	0.04
Thymol blue	0.04
Agar	15

Final pH 8.6±0.2

Dissolve89 gms in 1000 ml distilled water,heat to boiling to dissolve the medium completely.after cooling to 50˚c ,pour into petriplates.

4.Selenite F broth

Sodium hydrogen selenite	4 g
Peptone	5 g
Lactose	4 g
Disodium hydrogen phosphate	9.5 g
Sodium dihydrogen phosphate	0.5 g
Sterile water	1 litre

All the ingredients were dissolved in sterile water with sterile precautions. The yellow solution in about 10ml amounts distributed into screw capped universal bottles. Steamed for 20 minutes at 100˚c. Adjust pH to 7.1.

5.Mueller- Hinton Agar:

Beef extract	20 gm
Acidicase peptone	7.5 gm
Starch	1.5 gm
Agar	17 gm
Distilled water	1000 ml

The ingredients were dissolved in one liter of distilled water, mixed thoroughly. Heated with frequent agitation and boiled for 1 minute. pH adjusted to 7.4. Sterilized by autoclaving and poured in plates.

IDENTIFICATION OF THE ORGANISM :-

Organism	TSI	Citrate	Indole	Oxidase	Catalase	Glucose	Lactose	Sucrose	Maltose	Mannitol	xylose	arabinose	urease	Motility
Escherichia coli	A/A	Not utilised	+	-	+	AG	A	-	A	A	A	A	-	+
Shigella dysenteriae	K/A	Not utilised	-	-	+	A	-	-	-	-			-	-
Shigella flexneri	K/A	Not utilised	-	-	+	A	-	-	-	A			-	-
Shigella boydii	K/A	Not utilised	-	-	+	A	-	-	-	A			-	-
Shigella sonnei	K/A	Not utilised	-	-	+	A	Late	-	-	A			-	-
Salmonella typhi	K/A	Not utilised	-	-	+	A	-	-	A	A	A	-	-	+
Salmonella paratyphi A	K/A	Not utilised	-	-	+	AG	-	-	A	A	-	A	-	+
Salmonella paratyphi B	K/A	utilised	-	-	+	AG	-	-	A	A	A	A	-	+
Salmonella typhimurium	K/A	utilised	-	-	+	AG	-	-	A	A	A	A	-	+
Vibrio cholerae	A/A	utilised	+	+	+	A	-	A	A	A		-	-	+
Klebsiella Pneumoniae	A/A	Utilised	-	-	+	AG	A	A	A	A	+	+	+	-
Klebsiella oxytoca	A/A	Utilised	+	-	+	AG	A	A	A	A	+	+	+	-
Proteus. species	A/Awith H2S	v	V	-	+	AG	-	V	v	+			+	+
Pseudomonas .aeruginosa	K/No change	V	-	+	+	-	-	-	-	-			-	+

Note: A/A = Acid slant / Acid butt, + = Positive, - = Negative

A=Acid production, AG=Acid and gas production

ZONE SIZE INTERPRETATIVE CHART ACCORDING TO CLSI GUIDELINES-Kirby-Bauer Chart

Sl. No.	Drug	Disk Content mcg	Resistant mm or less	Intermediate mm	Sensitive mm or more
For GNB 1	Ampicillin	10	13 mm	14-16 mm	17 mm
2	Co-trimoxazole	1.25/3.75	10	11-15	16
5	Ciprofloxacin	5	15	16-20	21
6	Gentamycin	10	12	13-14	15
7	Cefotaxime	30	14	15-22	23
8	Tetracycline	30	11	12-14	15
9	Chloramphenicol	30	12	13-17	18
11	Amoxycillin – Clavulanic acid	20/10	13	14-17	18

APPENDIX III:

Reagents required for ELISA :

- Microwell plate, coated with monoclonal antibodies (mouse) against rotaviruses
- Sample-dilution buffer,
- Wash buffer, phosphate-buffered NaCl solution (10-fold concentrate);
- Positive control Inactivated monkey rotavirus (SA-11);
- Conjugate : Peroxidase-conjugated monoclonal antibody (mouse) against rotaviruses in stabilised protein solution;
- Substrate :Urea peroxide/TMB;
- Stop reagent, 1 N sulphuric acid
- Distilled or deionised water
- Test tubes
- Disposable pipettes
- Vortex mixer
- Micropipette for 50 -100 µl and 1 ml volume
- Measuring cylinder (1000 ml)
- Stop clock, Washing unit for mictrotitration pipettes or multichannel pipettes (300 µl)
- Photometer for microwell plates (450 nm and reference filter ≥ 600 nm where necessary)
- Filter paper (laboratory towels)
- Waste container containing 0.5 % hypochlorite solution

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Master chart

STUDY GROUP																		
Sl. No.	Name	age	sex	stools durn	freq	vomiting	frequency	abd pain	fever	breast feed	dehyd	consis	bloody	parasite	isolate/s	antimicrobial sensitivity	rota ELISA	GENOTYPE
1	prathipal	7 mths	mc	72Hrs st	7 to 10	>72hrs vom	4-6 epi	abd pain-	fev -	bf+	severe	watery	b-		V.cholerae	sen to tet,ampi,chlor res to cot	rotn eg	
2	muralidharan	9mths	mc	>72 hrs st	7 to 10	no vom		abd pain-	fev +	bf-	no	watery	b-	EH cyst	E.coli		rotn eg	
3	boominathan	10 mths	mc	48 hrs st	4 to 6	48hrs vom	1-3 epi	abd pain-	fev -	bf+	some	semi	b-		E.coli,Enterococci		rotn eg	
4	kaaviya	10mths	fc	72hrs st	>10	no vom		abd pain-	fev -	bf+	no	watery	b-		E.coli		rotn eg	
5	sanjay	8mths	mc	>72 hrs st	>10	48hrs vom	4-6 epi	abdpneg	fev +	bf+	severe	watery	b-		E.coli		rot pos	G1P[8]
6	mukesh	27mths	fc	>72 hrs st	7 to 10	no vom		abd pain-	fev -	bf+	no	semi	b-		S.aureus,entero		rotn eg	
7	elakiya	12mths	fc	48hrs st	>10	no vom		abd pain-	fev +	bf+	some	watery	b+		Ent.h'gic E.coli		rotn eg	
8	hariprakash	11mths	mc	72hrs st	>10	no vom		abd pain-	fev -	bf+	no	watery	b-		E.coli		rotn eg	

9	prabhu	15mths	mc	72hrs st	4 to 6	72hrs vom	1-3 epi	abdpneg	fev+	bf-	severe	watery	b-		E.coli		rotpos	G1P[8]
10	mercy	6mths	fc	48hrs st	7 to 10	48hrs vom	4-6 epi	abdpneg	fev+	**bf-**	some	watery	b-		enterococci		rotpos	G2P[4]
11	tamil	5mths	mc	>72 hrs st	4 to 6	no vom		abd pain-	fev-	bf+	no	watery	b-		S.aureus		rotneg	
12	Shalini	6mths	fc	48hrs st	7 to 10	24hrs vom	1-3 epi	abd pain-	fev+	bf+	some	watery	b-		E.coli		rotneg	
13	arshini	4.5 mths	fc	24hrs st	4 to 6	no vom		abd pain-	fev-	bf+	no	watery	b-		E.coli		rotneg	
14	kumaera	12mths	fc	24hrs st	>10	24hrs vom	4-6 epi	abd pain-	fev-	bf+	severe	semi	b+		E.coli		rotneg	
15	mukilan	8mths	mc	72hrs st	4 to 6	no vom		abdpneg	fev-	bf+	some	watery	b-		E.coli		rotpos	not typed
16	vinodhini	9mths	fc	48hrs st	4 to 6	24 hrs vom	1-3 epi	abd pain-	fev+	bf+	no	semi	b-		E.coli,K.oxy		rotneg	
17	deepika	9mths	fc	72hrs st	7 to 10	72hrs vom	1-3 epi	abdpneg	fev-	bf+	some	watery	b-		E.coli		rotpos	untypeable
18	jeyasri	8mths	fc	72hrs st	7 to	72hrs vom	1-3 epi	abd	fev+	bf+	some	semi	b-		k.oxy,E.coli		rotneg	

					10			pain-										
19	abdul aakif	12mths	fc	48hrs st	4 to 6	no vom		abd pain-	fev -	bf+	no	watery	b-		E.coli,Entero		rotneg	
20	kaavya	18mths	fc	72hrs st	4 to 6	72hrs vom	1-3 epi	abd pain-	fev -	bf-	some	watery	b-		E.coli		rotneg	
21	Faariya	18mths	fc	48 hrs st	7 to 10	48hrs vom	4-6 epi	abdpneg	fev +	bf-	severe	watery	b-		E.coli		rotpos	G1P[8]
22	monish	8mths	mc	48 hrs st	7 to 10	48hrs vom	1-3 epi	abd pain-	fev +	bf+	some	watery	b-		E.coli		rotneg	
23	rithik	15mths	mc	48 hrs st	>10	no vom		abd pain-	fev +	bf+	severe	watery	b-		E.coli		rotneg	
24	sahin banu	6mths	fc	48 hrs st	7 to 10	no vom		abd pain-	fev +	bf+	severe	watery	b-		E.coli		rotneg	
25	dharani	15mths	mc	48hrs st	4 to 6	48hrs vom	1-3 epi	abd pain-	fev +	bf+	no	watery	b+		E.coli,pseudo		rotneg	
26	ilakiyan	54mths	mc	24hrs st	4 to 6	no vom		abd pa+	fev +	bf+	no	watery	b+		E.coli,S.flexneri	sens to cip,gen,cefo,amclav,res to amp,cot	rotneg	
27	shunmuga priya	15mths	fc	24hrs st	>10	24hrs vom	1-3 epi	abd pain-	fev -	bf+	some	watery	b-		K.pneum,E.coli		rotneg	

28	vinthya	9mths	fc	48hrs st	4 to 6	48hrs vom	1-3 epi	abdpneg	fev+	bf+	no	watery	b-		E.coli		rotpos	not typed
29	tamilarasi	54mths	fc	48 hrs st	4 to 6	no vom		abd pain-	fev-	bf+	no	watery	b-	EH cyst	E.coli		rotneg	
30	jeganathan	10mths	mc	24hrs st	7 to 10	24hrs vom	4-6 epi	abd pain-	fev+	bf+	some	watery	b-		E.coli		rotneg	
31	prithika	5mths	fc	>72 hrsst	7 to 10	no vom		abd pain-	fev-	bf+	no	watery	b-		E.coli		rotneg	
32	valarnila	12mths	fc	24hrs st	>10	24hrs vom	4-6 epi	abdpneg	fev+	bf-	some	watery	b-		E.coli		rotpos	not typed
33	Aashika	17mths	fc	72hrs st	4 to 6	48hrs vom	1-3 epi	abd pain-	fev+	bf-	no	watery	b+		E.coli		rotneg	
34	Rohit	11mths	mc	48 hrs st	>10	48hrs vom	1-3 epi	abd pain-	fev+	bf-	some	watery	b+		Enth'gic.coli		rotneg	
35	mukesh	5mths	mc	24hrs st	4 to 6	24hrs vom	1-3 epi	abd pain-	fev-	bf+	no	semi	b-		E.coli,Pseudo		rotneg	
36	shyamala	7mths	fc	48 hrs st	4 to 6	no vom		abd pain-	fev-	bf+	no	semi	b-		K.pneum,E.coli		rotneg	
37	sumaiya	9mths	fc	48 hrs	7 to	48hrs vom	4-6 epi	abdp	fev	bf+	severe	watery	b-		E.coli		rotpos	not typed

				st	10			neg	+									
38	nithin	11mths	mc	72hrs st	>10	24hrs vom	4-6 epi	abd pain-	fev +	bf+	some	watery	b-		pseudo,E.coli		rotneg	
39	padmanaban	15mths	mc	>72hrss t	7 to 10	>72hrs vom	4-6 epi	abd pain-	fev +	bf+	severe	watery	b-		E.coli		rotneg	
40	raksana	7mths	fc	48 hrs st	>10	no vom		abd pain-	fev -	bf -	no	semi	b-		E.coli,S.flexneri	sens to cip,gen,res to cefo,amclav, amp,cot	rotneg	
41	jayalakshmi	8mths	fc	>72 hrss t	4 to 6	24hrs vom	1-3 epi	abd pain-	fev +	bf+	no	watery	b-		E.coli		rotneg	
42	krishna	36mths	mc	>72 hrss t	7 to 10	72hrs vom	1-3 epi	abdpneg	fev -	bf+	some	watery	b-		E.coli		rot pos	not typed
43	swathy	12mths	fc	24hrs st	>10	48hrs vom	1-3 epi	abd pain-	fev +	bf+	severe	watery	b-		E.coli		rotneg	
44	lathifa begum	8mths	fc	48 hrs st	4 to 6	no vom		abd pain-	fev +	bf-	no	watery	b-		E.coli,S.flexneri	sens to cip,gen,cefo,amclav,res to amp,cot	rotneg	
45	thangamani	15mths	mc	24hrs st	4 to 6	24hrs vom	1-3 epi	abd pain-	fev +	bf-	no	watery	b-		E.coli		rotneg	
46	tamilselvan	12mths	mc	24hrs st	7 to 10	24hrs vom	4-6 epi	abd pain-	fev +	bf+	some	watery	b+		E.coli,S.flexneri	sens to cip,gen,res to cefo,amclav, amp,cot	rotneg	

47	yazhini	24mths	fc	24hrs st	4 to 6	24hrs vom	4-6 epi	abd pain-	fev +	bf-	no	watery	b-	EH cyst	E.coli		rotneg	
48	shek rehman	10mths	mc	72hrs st	4 to 6	>72hrs vom	1-3 epi	abdpneg	fev +	bf-	some	watery	b-		E.coli		rotpos	not typed
49	srisanjev	8mths	mc	>72 hrsst	7 to 10	>72hrs vom	1-3 epi	abd pain-	fev -	bf+	severe	watery	b-		E.coli		rotneg	
50	nivedha	24mths	fc	>72 hrsst	7 to 10	no vom		abd pain-	fev +	bf-	some	watery	b-		E.coli,K.oxy		rotneg	
51	sabarish	12mths	mc	24hrs st	7 to 10	no vom		abd pa+	fev -	bf-	some	watery	b-	Giardia	E.coli		rotneg	
52	saravanan	36mths	mc	24hrs st	4 to 6	24hrs vom	1-3 epi	abd pa+	fev +	bf-	no	watery	b+		E.coli,S.flexneri	sens to cip,gen,cefo,amclav,res to amp,cot	rotneg	
53	tejes	8mths	fc	48 hrs st	7 to 10	48hrs vom	1-3 epi	abdpneg	fev +	bf-	severe	watery	b-		E.coli		rotpos	untypeable
54	rakesh	8mths	mc	24hrs st	7 to 10	24hrs vom	1-3 epi	abd pain-	fev -	bf+	some	watery	b-		E.coli		rotneg	
55	tamilarasan	30mths	mc	72hrs st	>10	no vom		abd pain-	fev +	bf-	some	watery	b+		E.coli		rotneg	
56	dharshini	54mths	fc	24hrs st	>10	no vom		abd	fev +	bf-	no	watery	b+		E.coli,S.dys	sens to cip,gen,cefo,am	rotneg	

								pa +								clav,res to amp,cot		
57	divyadarshini	24mths	fc	72hrs st	4 to 6	no vom		abd pa +	fev -	bf-	some	semi	b-		E.coli,S.flexneri	sens to cip,gen,res to cefo,amclav, amp,cot	rotneg	
58	monisha	12mths	fc	>72 hrsst	4 to 6	72hrs vom	4-6 epi	abd pain-	fev +	bf+	no	watery	b-		E.coli		rotneg	
59	Ezhilarasan	8mths	mc	72hrs st	4 to 6	72hrs vom	1-3 epi	abd pain-	fev +	bf-	some	watery	b-		E.coli		rotneg	
60	prithiv	16mths	mc	72hrs st	4 to 6	72hrs vom	1-3 epi	abdpneg	fev -	bf-	no	watery	b-		E.coli		rot pos	not typed
61	jithesh	12mths	mc	>72 hrsst	4 to 6	>72hrs vom	1-3 epi	abd pain-	fev -	bf+	no	semi	b-		E.coli		rotneg	
62	sreeharini	7mths	fc	>72 hrsst	4 to 6	no vom		abd pain-	fev +	bf+	no	watery	b-		E.coli,pseudo		rotneg	
63	vishali	8mths	fc	48 hrs st	4 to 6	48hrs vom	1-3 epi	abd pain-	fev +	bf+	no	watery	b-		E.coli,S.flexneri	sens to cip,gen,cefo,amclav,res to amp,cot	rotneg	
64	sanjay	36mths	mc	24hrs st	>10	24hrs vom	1-3 epi	abd pain-	fev +	bf-	severe	watery	b-		E.coli		rotneg	
65	dharani	7mths	fc	24hrs st	4 to 6	no vom		abd pain-	fev -	bf+	no	watery	b-		proteus,s.aureus		rotneg	

66	afra fathima	12mths	fc	>72 hrsst	7 to 10	no vom		abdpneg	fev-	bf+	some	watery	b-		E.coli		rot pos	G2P[4]
67	rajesh	8mths	mc	24hrs st	4 to 6	>72hrs vom	4-6 epi	abd pain-	fev+	bf-	no	watery	b-		E.coli		rotneg	
68	rahul	27mths	mc	72hrs st	>10	72hrs vom	1-3 epi	abd pain-	fev+	bf+	severe	watery	b-		E.coli		rotneg	
69	yamini	22mths	fc	72hrs st	7 to 10	72hrs vom	4-6 epi	abd pain-	fev-	bf-	no	semi	b-		E.coli		rotneg	
70	monisha	8mths	fc	24hrs st	7 to 10	no vom		abd pa+	fev-	bf-	no	watery	b-	cryptosporidium	E.coli		rotneg	
71	thannarasu	15mths	mc	24hrs st	7 to 10	24hrs vom	1-3 epi	abd pa+	fev+	bf-	no	watery	b-		S.flexneri	sens to cip,gen,cefo,amclav,res to amp,cot	rotneg	
72	suriya	12mths	mc	24hrs st	>10	24hrs vom	4-6 epi	abdpneg	fev+	bf-	some	watery	b-		K.oxy,		rot pos	not typed
73	aayisha siddika	42m	fc	>72 hrsst	7 to 10	no vom		abd pa+	fev+	bf+	some	semi	b+		E.coli,S.flexneri	sens to cip,gen,cefo,amclav,res to amp,cot	rotneg	
74	yuvaraj	21mths	mc	24hrs st	4 to 6	24hrs vom	1-3 epi	abd pain-	fev+	bf-	no	watery	b-		E.coli		rotneg	
75	gunal	10mths	mc	24hrs st	4 to	24hrs vom	4-6 epi	abd	fev-	bf+	no	watery	b-		k.oxy,E.coli		rotneg	

					6			pain-										
76	lathika	8mths	fc	72hrs st	4 to 6	24hrs vom	4-6 epi	abd pain-	fev -	bf+	severe	semi	b +		EH E.coli		rotn eg	
77	Dhanalakshmi	36mths	fc	>72 hrsst	4 to 6	48hrs vom	4-6 epi	abd pain-	fev +	bf-	no	watery	b-		E.coli		rotn eg	
78	jothi	36mths	fc	48 hrs st	4 to 6	no vom	4-6 epi	abd pa +	fev +	bf-	no	watery	b +	EH cyst	S.paratyphi A	sen to cip,cefo,amclav ,gen, cot,ampi	rotn eg	
79	Monish babu	36mths	mc	72hrs st	>10	48hrs vom	4-6 epi	abd pa +	fev +	bf-	some	watery	b-		k.oxy,E.coli		rotn eg	
80	Srikrishnan	9mths	mc	72hrs st	4 to 6	72hrs vom	7-10 epi	abd pain-	fev +	bf+	no	watery	b-		K.oxy		rotn eg	
81	Ashika	10mths	fc	24hrs st	4 to 6	no vom		abdpneg	fev -	bf-	some	watery	b-		E.coli		rot pos	not typed
82	Tamilarasu	11mths	mc	24hrs st	4 to 6	24hrs vom	1-3 epi	abd pain-	fev +	bf+	no	watery	b-		E.coli,k.oxy		rotn eg	
83	Maheswaran	12mths	mc	>72 hrs st	4 to 6	no vom		abd pain-	fev +	bf-	no	watery	b+		S.flexneri,E .coli	sens to cip,gen,cefo,amclav,res to amp,cot	rotn eg	
84	Iniya	15mths	fc	72hrs st	4 to 6	72hrs vom	1-3 epi	abd pain-	fev -	bf-	no	watery	b-		E.coli		rotn eg	

85	Akshara	15mths	fc	>72 hrs st	4 to 6	no vom		abd pa+	fev+	bf-	some	watery	b+		E.coli		rotneg	
86	Sanjana	7mths	fc	72hrs st	4 to 6	no vom		abd pain-	fev-	bf+	no	watery	b-		K.oxy		rotneg	
87	Thiyagarajan	9mths	mc	>72 hrs st	4 to 6	24hrs vom	1-3 epi	abd pain-	fev+	bf+	some	watery	b-		E.coli		rotneg	
88	Joefelix	8mths	mc	24hrs st	7 to 10	24hrs vom	1-3 epi	abdpneg	fev+	bf-	no	watery	b-		E.coli		rot pos	untypeable
89	Gokulesh	36mths	mc	>72 hrs st	4 to 6	no vom		abd pa+	fev-	bf+	some	watery	b-	Giardia	E.coli		rotneg	
90	Shalini	6mths	fc	48hrs st	4 to 6	24hrs vom	1-3 epi	abd pain-	fev-	bf-	no	watery	b-		E.coli,K.oxy		rotneg	
91	Kaavya	10mths	fc	>72 hrs st	7 to 10	no vom		abd pain-	fev+	bf-	no	watery	b-		pseudo,E.coli		rotneg	
92	Mohanakrishnan	9mths	mc	72hrs st	7 to 10	48hrs vom	1-3 epi	abd pain-	fev+	bf-	some	watery	b-		E.coli		rotneg	
93	Dharshan	6mths	mc	72hrs st	4 to 6	>72hrs vom	1-3 epi	abdpneg	fev-	bf+	no	watery	b-		E.coli		rot pos	G10P[untypeable]
94	Divya	36mths	fc	>72 hrs	7 to	no vom		abd	fev-	bf-	no	watery	b-	cryptosporidium	K.oxy		rotneg	

				st	10			pa +										
95	Nithilan	18mths	mc	>72 hrs st	>10	no vom		abd pa+	fev+	bf-	some	watery	b+		E.coli,S.flexneri	sens to cip,gen,cefo,amclav,res to amp,cot	rotneg	
96	Mohan kumar	7.5mths	mc	48hrs st	4 to 6	no vom		abd pa+	fev+	bf+	severe	watery	b-		E.coli,K.oxy		rotneg	
97	Mani	8mths	mc	72hrs st	4 to 6	no vom		abd pain-	fev+	bf-	no	watery	b-		E.coli		rotneg	
98	Agilesh	8mths	mc	72hrs st	4 to 6	48hrs vom	1-3 epi	abd pain-	fev-	bf-	some	watery	b-		Proteus		rotneg	
99	Jeeva	36mths	mc	48hrs st	4 to 6	no vom		abd pa+	fev+	bf+	no	watery	b-		entero,E.coli		rotneg	
100	Sriram	18mths	mc	48hrs st	4 to 6	no vom		abd pain-	fev+	bf+	no	watery	b-		E.coli		rotneg	
101	Tamil sekuvara	14mths	mc	48hrs st	4 to 6	no vom		abd pain-	fev-	bf+	no	watery	b-		E.coli		rotneg	
102	Lokesh	18mths	mc	>72 hrs st	4 to 6	72hrs vom	1-3 epi	abd pain-	fev-	bf+	no	watery	b-		pseudo,E.coli		rotneg	
103	Prabakaran	24mths	mc	48hrs st	7 to 10	48hrs vom	4-6 epi	abdpneg	fev+	bf-	some	watery	b-		E.coli		rotpos	G1P[8]

104	Vignesh	18mths	mc	>72 hrs st	>10	>72hrs vom	4-6 epi	abd pain-	fev+	bf-	some	watery	b-		E.coli		rotneg	
105	Naren bose	18mths	mc	24hrs st	4 to 6	24hrs vom	1-3 epi	abd pain-	fev+	bf+	no	watery	b-		E.coli		rotneg	
106	Kamalesh	11mths	mc	72hrs st	4 to 6	no vom		abd pain-	fev-	bf-	no	semi	b+		S.flexneri,E.coli	sens to cip,gen,cefo,amclav,res to amp,cot	rotneg	
107	Sanjay	36mths	mc	>72 hrs st	4 to 6	no vom		abd pa+	fev+	bf-	no	watery	b-		E.coli,entero		rotneg	
108	afra fathima	12mths	fc	>72 hrs st	7 to 10	no vom		abd pain-	fev-	bf-	severe	watery	b-		E.coli		rotneg	
109	yuvaraj	21mths	mc	24hrs st	4 to 6	24hrs vom	1-3 epi	abd pain-	fev+	bf-	no	watery	b-		proteus		rotneg	
110	gunal	10mths	mc	24hrs st	7 to 10	24hrs vom	4-6 epi	abdpneg	fev+	bf+	no	watery	b-		k.oxy		rot pos	not typed
111	rahul	12mths	mc	72hrs st	7 to 10	no vom		abd pa+	fev+	bf-	severe	semi	b+		S.flexneri,E.coli	sens to cip,gen,cefo,amclav,res to amp,cot	rotneg	
112	Abudali	51mths	mc	24hrs st	7 to 10	24hrs vom	4-6 epi	abd pain-	fev+	bf+	some	watery	b-	EH cyst	E.coli		rotneg	
113	Katheeja suhaina	24mths	fc	48hrs st	4 to	no vom		abd	fev+	bf-	no	watery	b-		E.coli		rotneg	

					6			pain-										
114	Dhanush	24mths	mc	48hrs st	4 to 6	no vom		abd pain-	fev +	bf-	no	watery	b-		E.coli		rotneg	
115	Santhosh	18mths	mc	>72 hrs st	4 to 6	>72hrs vom	4-6 epi	abd pain-	fev +	bf+	some	watery	b-		E.coli		rotneg	
116	Bavani	24mths	fc	48hrs st	>10	24hrs vom	1-3 epi	abd pain-	fev +	bf-	no	watery	b-		k.oxy,E.coli		rotneg	
117	Jaidev	9mths	mc	24hrs st	4 to 6	no vom		abd pain-	fev +	bf+	no	watery	b-		E.coli		rotneg	
118	Manoj	7mths	mc	>72 hrs st	7 to 10	no vom		abdpneg	fev -	bf+	some	watery	b-		E.coli		rot pos	not typed
119	Jayananthini	18mths	fc	72hrs st	7 to 10	72hrs vom	4-6 epi	abd pain-	fev +	bf-	no	watery	b-		E.coli		rotneg	
120	Valanshika	12mths	fc	72hrs st	4 to 6	no vom		abd pain-	fev +	bf-	no	watery	b-		E.coli		rotneg	
121	Balaji	18mths	mc	24hrs st	4 to 6	no vom		abd pain-	fev -	bf+	no	semi	b-		E.coli		rotneg	
122	Angel christina	12mths	fc	>72 hrs st	7 to 10	72hrs vom	4-6 epi	abd pa+	fev +	bf-	no	watery	b+		S.typhimurium	sen to cip,cefo,amclav,gen, cot res to ampi	rotneg	

123	Tarun	18mths	mc	72hrs st	>10	24hrs vom	4-6 epi	abdpneg	fev+	bf+	severe	watery	b-		E.coli		rotpos	not typed
124	Mani	8mths	mc	72hrs st	4 to 6	24hrs vom	4-6 epi	abd pain-	fev+	bf-	no	watery	b-		proteus,E.coli		rotneg	
125	Jacob	7mths	mc	24hrs st	4 to 6	24hrs vom	4-6 epi	abd pain-	fev-	bf+	no	watery	b-		E.coli		rotneg	
126	Praveen kumar	18mths	mc	48hrs st	7 to 10	no vom		abd pain-	fev-	bf-	no	watery	b-		E.coli		rotneg	
127	vijoovan	6mths	mc	>72 hrs st	4 to 6	no vom		abd pain-	fev+	bf+	some	semi	b-		k.oxy.E.coli		rotneg	
128	Sasikumar	54mths	mc	72hrs st	4 to 6	24hrs vom	4-6 epi	abd pain-	fev+	bf+	no	watery	b-	Ascaris egg	k.oxy		rotneg	
129	Vishwa	18mths	mc	24hrs st	4 to 6	24hrs vom	4-6 epi	abd pain-	fev-	bf+	severe	watery	b-		E.coli		rotneg	
130	Srinivas	48mths	mc	24hrs st	4 to 6	no vom		abd pain-	fev-	bf-	some	semi	b-		E.coli,proteus		rotneg	
131	Akash	18mths	mc	24hrs st	4 to 6	24hrs vom	1-3 epi	abd pain-	fev-	bf-	no	semi	b-		E.coli		rotneg	
132	Ayyanaar	32mths	mc	24hrs st	4 to	no vom		abd	fev-	bf+	no	watery	b-		pseudo,E.coli		rotneg	

					6			pain-										
133	Mohammed Farook	11mths	mc	72hrs st	7 to 10	72hrs vom	4-6 epi	abdpneg	fev+	bf+	severe	watery	b-		E.coli		rotpos	G1P[8]
134	Sri sanjeev	8mths	mc	>72 hrs st	7 to 10	>72hrs vom	4-6 epi	abd pain-	fev-	bf+	no	watery	b-		k.oxy,entero		rotneg	
135	sree	15mths	mc	48hrs st	4 to 6	no vom		abd pa+	fev+	bf-	no	semi	b-		E.coli		rotneg	
136	Shek rehman	10mths	mc	72hrs st	7 to 10	72hrs vom	1-3 epi	abd pain-	fev-	bf+	severe	semi	b-		entero,E.coli		rotneg	
137	Nivedha	24mths	fc	>72 hrs st	7 to 10	no vom		abd pain-	fev+	bf-	some	watery	b-		E.coli		rotneg	
138	Naresh kumar	21mths	mc	>72 hrs st	4 to 6	72hrs vom	1-3 epi	abd pain-	fev+	bf-	no	watery	b-		E.coli		rotneg	
139	Rithika	18mths	fc	24hrs st	4 to 6	no vom		abd pain-	fev+	bf+	no	watery	b-		k.oxy		rotneg	
140	Sylvia	9mths	fc	48hrs st	4 to 6	48hrs vom	1-3 epi	abd pain-	fev+	bf+	severe	watery	b-		EH E.coli		rotneg	
141	Muvin kumar	7mths	mc	48hrs st	4 to 6	no vom		abd pain-	fev-	bf+	no	watery	b-		E.coli		rotneg	

142	Sahistha	10mths	fc	48hrs st	4 to 6	no vom		abd pain-	fev -	bf+	severe	watery	b-		S.aureus,E.coli		rotneg	
143	Vandhana	24mths	fc	24hrs st	4 to 6	24hrs vom	1-3 epi	abd pa+	fev -	bf-	no	watery	b+		S.flexneri	sens to cip,gen,cefo,amclav,res to amp,cot	rotneg	
144	Doyal	12mths	mc	24hrs st	7 to 10	24hrs vom	4-6 epi	abdpneg	fev +	bf-	no	watery	b-		E.coli		rotpos	G1P[8]
145	David jebaselvan	12mths	mc	48hrs st	4 to 6	48hrs vom	1-3 epi	abd pain-	fev +	bf+	no	semi	b-		pseudo,E.coli		rotneg	
146	Uday	9mths	mc	72hrs st	4 to 6	no vom		abd pain-	fev -	bf+	severe	watery	b-		E.coli		rotneg	
147	vijayakumari	12mths	fc	24hrs st	4 to 6	no vom		abd pa+	fev +	bf-	no	semi	b-		k.oxy,E.coli		rotneg	
148	pooja sree	18mths	fc	48hrs st	7 to 10	no vom		abd pa+	fev +	bf+	no	watery	b-		E.coli		rotneg	
149	swathi	9mths	fc	>72 hrs st	4 to 6	>72hrs vom	1-3 epi	abd pain-	fev +	bf+	no	watery	b-		E.coli		rotneg	
150	jothi	24mths	fc	48hrs st	4 to 6	48hrs vom	4-6 epi	abd pain-	fev +	bf+	no	watery	b-		E.coli		rotneg	

CONTROLS						
SL. NO	AGE	SEX	BREAST FEED	BACTERIA	PARASITE	ROTA VIRUS
1	6mths	mc	bf+	E.coli		rot neg
2	12mths	mc	bf+	E.coli,k.oxy		rot neg
3	18mths	fc	bf+	E.coli		rot neg
4	15mths	fc	bf-	E.coli,pseudo		rot neg
5	30mths	mc	bf+	k.oxy	EH cyst	rot neg
6	7mths	mc	bf+	E.coli		rot neg
7	8mths	fc	bf+	E.coli,k.oxy		rot neg
8	36mths	fc	bf+	k.oxy		rot neg
9	18mths	fc	bf+	E.coli		rot neg
10	9mths	mc	bf+	E.coli		rot neg
11	6mths	fc	bf+	E.coli.k.oxy		rot neg
12	32mths	mc	bf+	k.oxy		rot neg
13	24mths	mc	bf+	E.coli		rot neg
14	12mths	fc	bf+	E.coli		rot neg
15	24mths	fc	bf+	pseudo		rot neg
16	50mths	mc	bf-	E.coli	EH cyst	rot neg
17	8mths	mc	bf+	E.coli		rot neg
18	21mths	mc	bf+	E.coli		rot neg
19	36mths	mc	bf-	k.oxy		rot neg
20	6mths	mc	bf+	E.coli		rot neg
21	12mths	mc	bf+	E.coli,entero		rot neg
22	30mths	mc	bf-	k.oxy		rot neg
23	24mths	fc	bf+	E.coli,k.oxy		rot neg
24	9mths	fc	bf+	E.coli		rot neg
25	36mths	fc	bf-	k.oxy		rot neg
26	12mths	mc	bf+	E.coli		rot neg
27	12mths	fc	bf+	E.coli,k.oxy		rot neg
28	40mths	mc	bf-	E.coli	EH cyst	rot neg
29	9mths	fc	bf+	E.coli		rot neg

30	18mths	mc	bf+	E.coli,proteus		rot neg
31	7mths	mc	bf+	E.coli		rot neg
32	7.5mths	fc	bf+	E.coli		rot neg
33	32mths	mc	bf+	pseudo		rot neg
34	24mths	mc	bf-	E.coli,k.oxy		rot neg
35	12mths	mc	bf+	E.coli		rot neg
36	9mths	fc	bf+	E.coli		rot neg
37	18mths	fc	bf-	S.aureus		rot neg
38	8mths	mc	bf+	E.coli		rot neg
39	24mths	mc	bf+	E.coli		rot neg
40	8.5mths	mc	bf+	E.coli		rot neg
41	42mths	fc	bf-	proteus		rot neg
42	12mths	fc	bf+	E.coli,k.oxy		rot neg
43	9mths	mc	bf+	E.coli		rot neg
44	8mths	fc	bf+	E.coli		rot neg
45	54mths	mc	bf-	entero	EH cyst	rot neg
46	32mths	fc	bf+	E.coli		rot neg
47	18mths	mc	bf+	E.coli		rot neg
48	12mths	fc	bf+	E.coli,k.oxy		rot neg
49	24mths	fc	bf+	E.coli		rot neg
50	9mths	mc	bf-	k.oxy		rot neg

Key to Master chart

Key to Master chart

mc	-	male child
fc	-	female child
bf	-	breast feeding status
fev	-	fever
abd pain	-	abdominal pain
vom	-	vomiting
b	-	blood stained
E.coli	-	Escherichia coli
K.oxy	-	Klebsiella oxytoca
K.pneum	-	Klebsiella pneumoniae
Pseudo	-	Pseudomonas sp
Entero	-	Enterococci
S.aureus	-	Staphylococcus aureus
V.cholerae	-	Vibrio cholerae
EH cyst	-	Entamoeba histolytica
Giardia	-	Giardia lamblia
Cryptosporidium	-	Cryptosporidium parvum
Rot	-	Rota virus antigen
cip	-	ciprofloxacin
gen	-	gentamycin
cefo	-	cefotaxime
amclav	-	amoxy clavulanic acid
amp	-	ampicillin
cot	-	cotrimoxazole
tet	-	tetracycline
chlor	-	chloramphenicol
sen	-	sensitive,
res	-	resistant

Proforma

PROFORMA

Name :

Age :

Sex : male/female

Address :

OP/IP No :

Micro ID No :

Complaints :

Loose stools :

Nature of stool : watery/bloody/mucus

Frequency : 4-6/7-10/>10 episodes/day

Duration : 24 hrs/48 hrs/72 hrs/>72 hrs

Vomiting :

Duration :

Abdominal pain :

Duration :

Fever :

Duration :

Cough/running nose:

Duration :

Difficulty in feeding:

Duration :

Coinfections :

Liver disease/Malignancy

Haematological abnormalities

Immunodeficiency/Steroids

Renal disease /Meningitis/Septicaemia

Mode of transmission:

Food borne(milk,meat,vegetables)

Fruits & beverages, icecream , sandwich

Water borne:

Person to person(contact)

Animal to human:(milk,undercooked meat)

Community acquired:

Past history :

Treatment history : Antibiotics/laxatives

General examination :

Dehydration: mild/moderate/severe

Systemic examination:

CVS:

RS:

Abdomen:

CNS: